(51) International Patent Classification 6 :	A2	(11) International Publication Number: **WO 99/06834**
G01N 33/53		(43) International Publication Date: 11 February 1999 (11.02.99)

(54) Title: METHODS FOR IDENTIFYING LIGAND SPECIFIC BINDING MOLECULES

(57) Abstract

The present invention provides a method for identifying a binding molecule having selective affinity for a ligand. The method consists of selectively immobilizing a diverse population of binding molecules to a solid support, simultaneously contacting the diverse population immobilized on the solid support with two or more ligands and determining at least one binding molecule which selectively binds to one or more of the ligands. The invention additionally provides a method for identifying an antibody having selective affinity for a tumor antigen. The method consists of selectively immobilizing a diverse population of antibodies to a solid support, simultaneously contacting the diverse population immobilized on the solid support with two or more tumor antigens and determining at least one antibody which selectively binds to one or more of the tumor antigens. The invention also provides an isolated binding polypeptide selective for a tumor antigen. Further provided by the present invention is a Complementarity Determining Region (CDR) or functional fragment thereof of an antibody selective for a tumor antigen.

1

METHODS FOR IDENTIFYING LIGAND SPECIFIC BINDING MOLECULES

This application claims the benefit of priority
of United States Serial No. 08/905,825, filed August 4,
1997, which was converted to a United States Provisional
5   Application, the entire contents of which is incorporated
herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates generally to
tumor cell therapy and more specifically to methods of
10   identifying new tumor specific binding molecules and
tumor specific antigens.

Recent years have provided tremendous progress
in the understanding of cancer development and
progression. Despite this increased knowledge, only
15   marginal decreases in death rates from most types of
cancer have been observed. The success rate of cancer
therapy is increased with early diagnosis. Cancer
therapy generally involves treatment with therapeutic
agents that affect not only cancer cells but other cells
20   in the body as well, often leading to debilitating side
effects. Thus, identification of tumor-specific
targeting agents, such as antibodies that bind to tumor
antigens, would provide reagents useful for earlier
diagnosis of specific types of cancers as well as
25   tumor-specific targeting agents for cancer therapy that
minimizes impact on non-tumor tissues.

Although some tumor-specific antigens have been
identified, obtaining useful tumor-specific targeting
agents has remained elusive. Of interest for therapeutic
30   purposes are human antibodies that can be used to target
toxins to specific types of tumors. The advantage of
using human antibodies is that they are least likely to

2

cause an immune response that would remove the antibody
and toxin from the body during cancer therapy. However,
development of human antibodies capable of targeting
specific tumors has proven difficult.

5   To obtain antibodies or other agents capable of
specifically targeting tumor types, a screen of a large
number of possible agents is required. For example, to
obtain a monoclonal antibody specific for a tumor
antigen, it is necessary to generate and screen tens or
10 hundreds of hybridoma cell lines. The process is
laborious, time consuming and additionally requires the
initial purification or preparation of the antigen or
tumor cell.

         A number of other screening approaches have now
15 been developed, including recombinant methods utilizing
bacteria and yeast, that allow identification of specific
binding partners for a particular molecule of interest.
For example, it is now possible to produce large display
or combinatorial libraries of antibodies or other types
20 of binding molecules. However, these methods generally
require the screening of the libraries using one or more
relatively purified molecules of interest.

         Regardless of the available screening
approaches, the identification of tumor specific antigens
25 and their subsequent isolation has proven difficult over
the years. Methods which have attempted to circumvent
this problem through the use of cell lysates to screen
for specific binding molecules has unfortunately resulted
in molecules which exhibit inadequate binding
30 specificities. Therefore, the availability of specific
binding molecules which can be adapted to cancer
diagnosis and therapy has been inherently lacking.

3

Thus, there exists a need for rapid and efficient methods to identify specific binding molecules to tumor antigens. The present invention satisfies this need and provides related advantages as well.

5   SUMMARY OF THE INVENTION

The present invention provides a method for identifying a binding molecule having selective affinity for a ligand. The method consists of selectively immobilizing a diverse population of binding molecules to
10 a solid support, simultaneously contacting the diverse population immobilized on the solid support with two or more ligands and determining at least one binding molecule which selectively binds to one or more of the ligands. The invention additionally provides a method
15 for identifying an antibody having selective affinity for a tumor antigen. The method consists of selectively immobilizing a diverse population of antibodies to a solid support, simultaneously contacting the diverse population immobilized on the solid support with two or
20 more tumor antigens and determining at least one antibody which selectively binds to one or more of the tumor antigens. The invention also provides an isolated binding polypeptide selective for a tumor antigen. Further provided by the present invention is a
25 Complementarity Determining Region (CDR) or functional fragment thereof of an antibody selective for a tumor antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the optimization of human Fab
30 binding molecule immobilization on a solid support.

4

Figure 2 shows the identification of human Fab binding molecules to tumor antigens using binding molecules selectively immobilized on a solid support and compares detection efficiencies to binding molecules

5   directly immobilized on a solid support.

Figure 3 shows the increased binding specificity and decreased background of detection using selective immobilization of human Fab binding molecule populations on a solid support compared to direct

10   immobilization.

Figure 4 shows the binding specificity of human Fab binding molecules identified by selective immobilization on a solid support to tumor cell monolayers.

15   Figure 5 shows fluorescent activated cell sorting of tumor cells with human Fab binding molecules identified by selective immobilization on a solid support.

DETAILED DESCRIPTION OF THE INVENTION

20   The invention provides rapid and efficient methods for the identification of binding molecules which exhibit selective affinity for one or more ligands of interest. The methods are advantageous in that they allow the simultaneous screening of multiple binding

25   molecules against multiple ligands of interest. Moreover, very little information is required regarding the identity or function of either the binding molecule or the ligand. For example, diverse populations of binding molecules can be simultaneously screened against

30   diverse populations of ligands to rapidly identify

5

numerous molecules exhibiting a desired binding
specificity. The methods of the invention can therefore
be advantageously applied for the discovery of specific
reagents for diagnosis and treatment of human diseases.

5   As used herein, the term "binding molecule" is
intended to refer to a molecule of sufficient size and
complexity so as to be capable of selectively binding a
ligand. Such molecules are generally macromolecules,
such as polypeptides, nucleic acids, carbohydrate or
10 lipid. However, derivatives, analogues and mimetic
compounds as well as small organic compounds are also
intended to be included within the definition of this
term. The size of a binding molecule is not important so
long as the molecule exhibits or can be made to exhibit
15 selective binding activity to a ligand. For example, a
binding molecule can be as little as about one or two,
and as many as tens or hundreds of monomer building
blocks which constitute a macromolecule binding molecule.
Similarly, an organic compound can be a simple or complex
20 structure so long as selective binding affinity can be
exhibited.

Binding molecules can include, for example,
antibodies and other receptor or ligand binding
polypeptides of the immune system. Such other molecules
25 of the immune system include for example, T cell
receptors (TCR), major histocompatibility complex (MHC),
CD4 receptor, and CD8 receptor. Additionally, cell
surface receptors such as integrins, growth factor
receptors and cytokine receptors, as well as cytoplasmic
30 receptors such as steroid hormone receptors are
substantially also included within the definition of the
term binding molecule. Furthermore, DNA binding
polypeptides such as transcription factors and DNA

6

replication factors are likewise included within the
definition of the term binding molecule. Finally,
polypeptides, nucleic acids and chemical compounds such
as those selected from random and combinational libraries
5   are also included within the definition of the term so
long as such a molecule exhibits or can be made to
exhibit selective binding activity toward a ligand.

As used herein, the term "polypeptide" when
used in reference to a binding molecule or a ligand is
10 intended to refer to peptide, polypeptide or protein of
two or more amino acids. The term is similarly intended
to refer to derivatives, analogues and functional
mimetics thereof.

As used herein, the term "ligand" refers to a
15 molecule that can be selectively bound by a binding
molecule. A ligand can be essentially any type of
molecule such as polypeptide, nucleic acid, carbohydrate,
lipid, or any organic derived compound. Those skilled in
the art know what is meant by the meaning of the term
20 ligand. Specific examples of ligands are the tumor
antigens described herein which are selectively bound by
the human antibody binding molecules described in the
examples.

As used herein, the term "diverse population"
25 is intended to refer to a group of two or more different
molecules. A diverse population of binding molecules can
have similar biochemical function as long as the function
or structure of the binding molecules are not identical.
A diverse population can include, for example, a
30 population of binding molecules that are antibodies
capable of recognizing the same or different ligands.
Moreover, the same binding molecule can recognize two

7

different ligands based on different ligand
conformations. In such cases, the binding molecules
would be considered distinct based on function and
multiple molecules of the same binding molecule would
5   therefore comprise a diverse population.

       As used herein, the term "selective" or
"selectively" when referring to the binding of a binding
molecule to a ligand or the immobilization of a
population to a solid support is intended to mean that
10   the interaction can be discriminated from unwanted or
non-specific interactions. Discrimination can be based
on, for example, affinity or avidity and therefore can be
derived from multiple low affinity interactions or a
small number of high affinity interactions. For example,
15   a binding molecule interaction with a ligand is generally
greater than about 10^{-4} M, is preferably greater than
about 10^{-5} M and more preferably greater than about 10^{-6} M.
High affinity interactions are generally greater than
about 10^{-8} M to 10^{-9} M or greater.

20   As used herein, the term "immobilizing" or
grammatical equivalents thereof, refers to the
attachment, as through the binding of a population of
binding molecules, to a solid support. Immobilization
can be through specific interactions with the binding
25   molecule and an agent on the solid support. The agent
can be, for example, a chemical moiety which allows
covalent or non-covalent interactions sufficient to hold
the population of binding molecules to the solid support.
Immobilization can also be through tethers or linkers.
30   Such linkers can be covalent linkers, hydrolyzable
linkers, photo-labile linkers or other linkers that allow
the binding molecules to be selectively attached.
Linkers can also be polypeptides or other biomolecular

8

linkers such as antibodies, lipid attachments,
streptavidin, receptors, fusion polypeptides, or any
biomolecule that can tether the binding molecule to the
solid support. Additionally, domains of polypeptides can

5   similarly be linkers. For example, hydrophobic domains
which allow direct absorption to a plastic due to
specific sequences which are molecular tags or
recognition sequences can be linkers for binding
polypeptides.

10   As used herein, the term "solid support" refers
to a solid medium which is sufficiently stable so as to
allow immobilization of a population of binding
molecules. Solid supports can include, for example,
membranes such as nitrocellulose, nylon, polyvinylidene

15   difluoride, plastic, glass, polyacrylamide or agarose.
Solid supports can also be made in essentially any size
or shape so long as it supports the immobilization of a
population of binding molecules. For example, the solid
support can be a flat planar surface such as a natural or

20   synthetic membrane filter or a glass slide.
Alternatively, the solid support can be of various
spherical shapes, including, for example, beads made of
glass, polyacrylamide or agarose. Porous mediums can
similarly be used as solid supports and such mediums are

25   included within the definition of the term as used
herein. Additionally, any of the solid supports can be
modified, for example, to include functional chemical
groups that can be used directly or indirectly for
attachment of binding molecules or linkers.

30   As used herein, the term "antibody" refers to a
polypeptide which binds to a ligand and is intended to be
used consistently with its meaning within the art. The
term immunoglobulin is similarly intended to fall within

9

the scope of the meaning of the term antibody as it is
known and used within the art. The polypeptide can be
the entire antibody or it can be any functional fragment
thereof which binds to the ligand. The meaning of the

5   term is intended to include minor variations and
modifications of the antibody so long as its function
remains uncompromised. Functional fragments such as Fab,
$F(ab)_2$, Fv, single chain Fv (scFv) and the like are
similarly included within the definition of the term

10   antibody. Such functional fragments are well known to
those skilled in the art. Accordingly, the use of these
terms in describing functional fragments of an antibody
are intended to correspond to the definitions well known
to those skilled in the art. Such terms are described

15   in, for example, Harlow and Lane, Antibodies: A
Laboratory Manual, Cold Spring Harbor Laboratory, New
York (1989); Molec. Biology and Biotechnology: A
Comprehensive Desk Reference (Myers, R.A. (ed.), New
York: VCH Publisher, Inc.); Huston et al., Cell

20   Biophysics, 22:189-224 (1993); Plückthun and Skerra,
Meth. Enzymol., 178:497-515 (1989) and in Day, E.D.,
Advanced Immunochemistry, Second Ed., Wiley-Liss, Inc.,
New York, NY (1990), which are incorporated herein by
reference.

25   As with the above terms used for describing
antibodies and functional fragments thereof, the use of
terms which reference other antibody domains, functional
fragments, regions, nucleotide and amino acid sequences
and polypeptides or peptides, is similarly intended to

30   fall within the scope of the meaning of each term as it
is known and used within the art. Such terms include,
for example, "heavy chain polypeptide" or "heavy chain",
"light chain polypeptide" or "light chain", "heavy chain
variable region" (V_H) and "light chain variable region"

10

(V_L) as well as the term "complementarity determining
region" (CDR).

In the case where there are two or more
definitions of a term which is used and/or accepted

5   within the art, the definition of the term as used herein
is intended to include all such meanings unless
explicitly stated to the contrary. A specific example is
the use of the term "CDR" to describe the non-contiguous
antigen combining sites found within the variable region

10   of both heavy and light chain polypeptides. This
particular region has been described by Kabat et al.,
U.S. Dept. of Health and Human Services, "Sequences of
Proteins of Immunological Interest" (1983) and by Chothia
et al., J. Mol. Biol. 196:901-917 (1987) and additionally

15   by MacCallum et al., J. Mol. Biol. 262:732-745 (1996),
which are incorporated herein by reference, where the
definitions include overlapping or subsets of amino acid
residues when compared against each other. Nevertheless,
application of either definition to refer to a CDR of an

20   antibody or variants thereof is intended to be within the
scope of the term as defined and used herein. The
appropriate amino acid residues which encompass the CDRs
as defined by each of the above cited references are set
forth below in Table I as a comparison. The exact

25   residue numbers which encompass a particular CDR will
vary depending on the sequence and size of the CDR.
Those skilled in the art can routinely determine which
residues comprise a particular CDR given the variable
region amino acid sequence of the antibody.

30   As used herein, the term "tumor antigen" refers
to a molecule that can be used as a marker to
discriminate or distinguish a tumor cell from a normal

11

Table I: CDR Definitions

		Kabat[1]	Chothia[2]	MacCallum[3]
V_H	CDR1	31-35	26-32	30-35
V_H	CDR2	50-65	52-56	47-58
V_H	CDR3	95-102	95-102	93-101
V_L	CDR1	24-34	24-34	30-36
V_L	CDR2	50-56	50-56	46-55
V_L	CDR3	89-97	89-97	89-96

[1] Residue numbering follows the nomenclature of Kabat et al., *supra*

[2] Residue numbering follows the nomenclature of Chothia et al., *supra*

[3] Residue numbering follows the nomenclature of MacCallum et al., *supra*

cell. Such discriminatory markers can manifest themselves in various ways such as preferential expression or unique cellular or subcellular localization in a tumor cell. A tumor cell for which such antigens preferentially mark can be any cell which undergoes abnormally frequent cell proliferation or unregulated survival. Such a cell can be a neoplastic cell which may or may not be fully characterized and classified as tumor or cancer cell.

The invention provides a method for identifying a binding molecule having selective affinity for a ligand. The method consists of: a) selectively immobilizing a diverse population of binding molecules to a solid support; b) simultaneously contacting the diverse population immobilized on the solid support with two or more ligands; and c) determining at least one binding molecule which selectively binds to one or more of the ligands.

12

The methods of the invention employ the selective immobilization of binding molecules to a solid support and the screening of the immobilized binding molecules for selective binding interactions against two

5 or more ligands of interest. Selective immobilization functions to increase the sensitivity of the binding interaction being measured since initial immobilization of a selected population of molecules onto a solid support reduces non-specific binding interactions with

10 irrelevant molecules or contaminants which can be present in the reaction. For example, the difficulty of measuring selective binding interactions increases with the complexity of the binding assay as well as the number and diversity of different species within the binding

15 reaction. The methods of the invention circumvent such difficulties since the selective immobilization of the binding molecule population substantially reduces the number of unwanted binding interactions through the removal of irrelevant molecules within the reaction.

20 Methods exist in the art for generating and screening populations of binding molecules against one or more purified ligands or targets of interest. However, when populations of binding molecules are screened against populations of ligands or complex compositions

25 such as cells or cell extracts, the identification of specific binding events is rare. The methods of the invention allow for the efficient screening of binding molecule populations against ligand populations for the rapid identification of selective binding events. The

30 methods of the invention provide improved sensitivity and specificity of detection through the selective immobilization of the binding molecule population on a solid support.

13

The methods of the invention are applicable for
both large and small populations of binding molecules
which can be either substantially purified or enriched
such as through separation of the population by affinity
5   techniques. Alternatively, such diverse populations can
be heterogeneous compositions such as those being
contained within a cell, cell extract or an *in vitro*
derived synthesis. Selective immobilization of such
populations provides sufficient enrichment of the
10   population so as to be applicable for screening with two
or more ligands of interest for selective binding
affinity.

The choice of the binding molecule population
will depend on the type of binding molecule desired, the
15   need and the ultimate intended use of the binding
molecule. For example, populations of binding molecules
can be produced or derived from essentially any source so
long as the population contains at least one binding
molecule which exhibits selective binding affinity for
20   the ligand or ligand composition of interest or so long
as the population is of sufficient diversity so that
there is a very likely probability that the population
will contain at least one molecule which will exhibit
selective binding to the ligand of interest.

25   At least two general approaches can be used for
obtaining binding molecule populations which will meet
the above criteria. One approach is to generate binding
molecule populations from molecules known to function as
binding molecules or known to exhibit or be capable of
30   exhibiting binding activity. A specific example of
molecules known to function as binding molecules or
exhibit binding activity include antibodies and other
receptors of the immune repertoire. The normal function

of such molecules is to bind essentially an infinite
number of different antigens and ligands. Therefore,
generating a diverse population of binding molecules from
an antibody repertoire, for example, will allow the

5   identification of a binding molecule against essentially
any desired ligand. Numerous other binding molecule
populations exist as well and are described further
below.

A second approach is to generate a large
10 population of unknown molecules. The population should
be generated to contain a sufficient diversity of
sequence or structure so as to contain a molecule which
will bind to the ligand composition of interest. An
advantage of this approach is that no prior knowledge of

15 sequence, structure or function is required. Instead,
all that is necessary is to generate a population of
sufficient size and complexity so that the population
will have a high probability of exhibiting a specific
binding interaction to the ligand complex by chance. A

20 specific example of such a population are random
libraries of peptides, nucleic acids and small molecule
compounds. Numerous other types of "random" library
molecule populations exist and are described further
below.

25   Prior knowledge of whether populations obtained
by either of the approaches described above contain at
least one binding molecule which exhibits selective
binding affinity for the ligand or composition of
interest is not necessary. Instead, all that is

30 necessary is to produce a population sufficiently diverse
for the intended need or purpose so as to likely contain
a binding molecule against the ligands or ligand
compositions of interest. Those skilled in the art will

know what size and diversity is necessary or sufficient
for the intended purpose.

As stated previously, the choice of which type
of binding molecule population to use will depend on the
5   specific need and desired use of the selected binding
molecule. For example, if binding molecules having high
affinity interaction are desired, then the use of a
population of antibody binding molecules can be
preferred. Other binding polypeptides and receptors of
10   the immunoglobulin superfamily of receptors additionally
can be used as well. Alternatively, if binding molecules
are desired for receptors, for example, it may be desired
to use populations of random peptide or nucleic acid
sequences. Specific examples would include, for example,
15   the use of random peptide populations to identify peptide
binding molecules specific for cell surface receptors,
random small molecule populations for the identification
of binding molecules having selective binding affinity to
cytoplasmic receptors such as steroid receptors, and
20   random nucleic acid populations for the identification of
binding molecules selective for nucleic acid binding
polypeptides. Those skilled in the art will know or can
determine what type of approach and what type of binding
molecule population is applicable for an intended purpose
25   and desired need.

The size and diversity of the binding molecule
population to use will depend on, for example, the ligand
population or ligand composition for which selective
binding molecules are desired as well as the number of
30   selective binding molecules desired, the range of desired
affinities and the time frame available for identifying
an appropriate binding molecule. For example, if the
application of the methods of the invention are for the

16

identification of one or a few binding molecules with
selective affinity for a ligand, then a limited number of
binding molecules can comprise the population to be
selectively immobilized on a solid support. Such a

5   limited number of binding molecules can be as small as
between about two to ten but will typically comprise tens
of molecules.

As the desired number of binding molecules to
be identified increases, so does the size and diversity

10 of the population. Similarly, as the range of desired
affinities and speed of identification of binding
molecules with selective affinity for a ligand increases,
so does the size and diversity of the binding molecule
population. Moreover, the size of the population of

15 binding molecules will similarly increase as the number
of ligands or complexity of the ligand population
increases for which selective binding molecules are
desired. Thus, as each of these factors increase, the
size and diversity of the binding molecule population can

20 similarly increase.

Moderate sized populations will consist of
hundreds and thousands of different binding molecules
within the population whereas a large sized binding
molecule population will consist of tens of thousands and

25 millions of different binding molecule species. More
specifically, large and diverse populations of binding
molecules for the identification of a binding molecule
will contain any of about 10^4, 10^5, 10^6, 10^7, 10^8, 10^9,
10^{10}, or more, different binding molecule species. One

30 skilled in the art will know the approximate diversity of
the population of binding molecules which will be
sufficient to identify the desired number of binding
molecules.

17

Recombinant libraries of binding molecules are
particularly advantageous for the methods of the
invention since large and diverse populations can be
rapidly generated and screened with two or more ligands
5   of interest. Binding molecule populations can be
directly used from the cell extracts of library
populations, for example. Methods employed for
selectively immobilizing the binding molecule populations
are discussed further below. Moreover, recombinant
10 libraries of expressed polypeptide or nucleic acid
products are applicable to the methods of the invention
since they can be engineered in a large number of
different ways so as to facilitate or directly function
in the selective immobilization of the binding molecule
15 population to a solid support. Recombinant library
methods similarly allow for the production of a large
number of binding molecule populations from naturally
occurring repertoires which inherently contain features
for selective immobilization of the population to a solid
20 support. Nevertheless, whether recombinant or otherwise,
essentially any source of binding molecule populations
can be used so long as the source provides a sufficient
size and diversity of different binding molecules
appropriate for the intended purpose and so long as the
25 population of binding molecules can be selectively
immobilized to a solid support.

Thus, the invention provides a method of
identifying a binding molecule having selective affinity
for a ligand wherein the binding molecule population is
30 produced by a recombinant library. Binding molecule
populations other than recombinant libraries exist as
well and can similarly be used for the screening and
identification of binding molecules having selective
affinity for a ligand.

18

A specific example of recombinant libraries as
sources of binding molecule populations are phage
expression libraries in which lysogenic phage cause the
release of bacterially expressed binding molecule
5 polypeptides. In another type of phage expression
library, large numbers of potential binding molecules can
be expressed as fusion polypeptides on the periplasmic
surface of bacterial cells. In both of these examples,
the expressed polypeptides are available for
10 immobilization on a solid support. Libraries in yeast
and higher eukaryotic cells exist as well and are
similarly applicable in the methods of the invention.
Those skilled in the art will know or can determine what
type of library is applicable for a specific purpose.

15   The invention also provides a method for
identifying a binding molecule selective for a ligand,
wherein the binding molecule is identified from an
antibody population.

As stated previously, there are at least
20 several approaches from which to obtain a starting
population of binding molecules to use for the
identification of a molecule having selective binding
affinity for a ligand. One approach is to use a
population of binding molecules that normally exhibit
25 binding function. Antibody binding molecules are
amenable to such an approach since they inherently
function as binding molecules and can be generated by
biological and recombinant methods to exhibit binding
specificities to essentially any antigen. Thus, the
30 populations of binding molecules of the invention can be
antibodies or functional fragments thereof.

19

Populations of antibody binding molecules can be generated by a variety of means known to those skilled in the art. For example, populations of antibody binding molecules can be generated from hybridoma technology and

5   populations of monoclonal antibodies can be used in the methods of the invention to identify a monoclonal antibody binding molecule, for example, having selective affinity for a ligand. Alternatively, large populations of antibody binding molecules can be expressed, for

10 example, by various recombinant methods known in the art.

Methods exist in the art to amplify, by polymerase chain reaction and other related methods known to those skilled in the art, essentially the entire antibody repertoire of a particular organism and express

15 as a recombinant population a diverse combination of the heavy and light antibody chains or functional fragments thereof. Organisms from which antibody repertoires can be derived include, for example, human, mouse, human, rabbit, goat, chicken or any organism capable of

20 producing antibodies. Functional fragments include, for example, Fab, Fv and CDR regions of the antibody molecules. The antibodies or functional fragments thereof can then be screened by selective immobilization onto a solid support. In the Examples described herein,

25 a human Fab fragment expression library was used to screen for binding molecules with selective affinity for tumor antigens.

In addition to antibody repertoires as starting sources for binding molecule populations having inherent

30 binding capabilities, the binding molecule populations can also be derived from other molecules of the immune system having known or inherent binding capabilities. For example, the binding molecules can be T cell

20

receptors (TCR). T cell receptors contain two subunits,
α and β, which are similar to antibody variable region
sequences in both structure and function. In this
regard, both subunits contain variable region which
5   encode CDR regions similar to those found in antibodies
(Immunology, Third Ed., Kuby, J. (ed.), New York, W.H.
Freeman & Co. (1997), which is incorporated herein by
reference). The CDR containing variable regions of TCRs
bind to antigens presented on the cell surface of
10   antigen-presenting cells and are capable of exhibiting
binding specificities to essentially any particular
antigen. As with antibody repertoires, there is
sufficient TCR sequence information known to allow the
amplification and recombinant engineering of essentially
15   the entire TCR repertoire. Thus, TCRs are amenable to
the methods of the invention since they inherently
function as binding molecules and can be generated by
biological and recombinant methods to exhibit binding
specificities to essentially any antigen.

20   Other exemplary binding molecules of the immune
system which exhibit known or inherent binding functions
include receptors of the major histocompatibility complex
(MHC), CD4 and CD8. MHC functions in mediating
interactions between antigen-presenting cells and
25   effector T cells. CD4 and CD8 receptors function in
binding interactions between effector T cells and
antigen-presenting cells. CD4 and CD8 have the
additional advantage in that they exhibit similar CDR
region structure as do antibodies and TCRs sequences.

30   Other binding molecules exhibiting known or
inherent binding function which are amenable for use as
starting populations in the methods of the invention
include a variety of receptors such as cell surface,

21

cytoplasmic and nuclear receptors. Specific example of
each of these classes of binding molecules will be
provided below. Binding molecules other than those
discussed below also exist and can be used in the methods
5   of the invention. Such other molecules are known or can
be determined by those skilled in the art using the
teachings provided herein.

        Integrins, for example, are cell surface
receptors involved in a variety of physiological
10 processes such as cell attachment, cell migration and
cell proliferation. Integrins mediate both cell-cell and
cell-extracellular matrix adhesion events. Structurally,
integrins consist of heterodimeric polypeptides where a
single α chain polypeptide noncovalently associates with
15 a single β chain. In general, different binding
specificities are derived from unique combinations of
distinct α and β chain polypeptides. For example,
vitronectin binding integrins contain the α_v integrin
subunit and include $\alpha_v\beta_3$, $\alpha_v\beta_1$ and $\alpha_v\beta_5$, all of which
20 exhibit different ligand binding specificities.

        Binding molecule populations can also be
produced from other cell surface receptors. These other
cell surface receptors include growth factor or hormone
receptors for ligands such as platelet derived growth
25 factor, epidermal growth factor, insulin, insulin-like
growth factor, hepatocyte growth factor, and other growth
factors and hormones. In addition, cell surface
receptors include cytokine receptors such as those for
the interleukins and interferons.

30   Cytoplasmic receptors can additionally be used
as the binding molecule population for use in the methods

22

of the invention. Cytoplasmic receptors include, for
example, the steroid hormone receptors such as the
thyroid hormone receptor, retinoic acid receptor,
glucocorticoid receptor, estrogen receptor and others.
5   In addition, binding molecule populations can be
generated from DNA or other nucleic acid binding
polypeptides that exhibit known or inherent binding
function in processes such as replication, transcription,
processing or translation.

10   As described previously, the binding molecule
populations can also be derived from random libraries of
unknown sequences or structures. In this approach it is
sufficient to produce a large and diverse library so as
to exhibit a likely probability that one or more
15 sequences within the binding molecule population will
exhibit selective binding affinity to the ligand of
interest. All that is necessary to practice the methods
of the invention using such populations is to be able to
selectively immobilize the random population of binding
20 molecules to a solid support. Methods are described
below for selectively immobilizing essentially any
population of binding molecules to a solid support.
Moreover, using the teachings described herein, those
skilled in the art will know how to apply other methods
25 so as to allow the selective immobilization of a
population of binding molecules to a solid support.

         Finally, the binding molecules can also be
synthetic compounds produced by, for example,
combinatorial chemistry methods known to those skilled in
30 the art. Such combinatorial chemical libraries can be
selectively immobilized onto a solid support and then
screened for the identification of molecules having
selective binding affinity for a ligand. Additionally,

23

large populations of other compounds, such as natural or
synthetic compound libraries are also equally applicable
as a binding molecule population so long as they can be
selectively immobilized onto a solid support.

5   The invention provides a method for identifying
a binding molecule exhibiting selective affinity for a
ligand from a diverse population of binding molecules.
The binding molecule is identified by simultaneously
contacting a diverse population of immobilized binding
10   molecules with two or more ligands.

           The methods of the invention allow for the
simultaneous screening of two or more ligands of
interest. Binding molecules exhibiting selective
affinity can be obtained to one or more of the ligands of
15   interest. The ligand population will be selected
depending on the need and intended use of the binding
molecule as well as the characteristics of the ligands or
ligand compositions. For example, using the methods of
the invention to selectively immobilize a population of
20   binding molecules, selective binders can be identified to
ligands as complex as tumor cells or specific tissue
types as well as to simple ligand populations of two or
more different species. An advantage of the method is
that sources of relatively crude ligand populations can
25   be used which facilitates the use of whole tissues, tumor
cells and cell lysates as ligand sources. In addition,
ligands which are not biochemically well characterized
can additionally be used in the methods of the invention.

           In the case where the ligand populations are
30   complex compositions such as tissues, cells, cell
lysates, and cellular or subcellular compartments, it is
the individual molecules which comprise each of these

24

complex compositions that make up the ligand population.
Therefore, by choosing a complex composition such as a
cell or cell lysate, for example, the ligand population
inherently contains two or more ligands that are used to
5   screen a population of binding molecules immobilized on a
solid support. Identifying a binding molecule selective
to at least one of the ligands within the population will
yield a binding molecule selective to the complex
composition itself. Thus, the methods of the invention
10 are applicable to the efficient identification of binding
molecules to, for example, tumor cells or other cellular
pathologies for the selective targeting of therapeutic
agents. The methods of the invention are similarly
applicable to the identification of binding molecules to
15 normal or diseased tissue for the selective targeting of,
for example, diagnostic agents such as imaging reagents.

In order to identify a binding polypeptide
having selective binding affinity to the ligand, it is
necessary to selectively immobilize a population of
20 binding molecules and then to contact the binding
molecules with two or more ligands. As described further
below, successful binding of a binding molecule within
the population indicates selective binding to at least
one of the ligands within the population. Selectivity
25 can be further confirmed by, for example, comparing the
binding affinity of the positive binder to a non-specific
or control ligand population. If desired, such a
comparison can be performed as a control during the
initial identification of the binding molecule such as
30 using normal cells and tumor cells as the control and
ligand populations, respectively. Alternatively, such a
comparison can be subsequently performed using either the
initial ligand populations or more purified form of
either the binding molecule or the ligand of interest.

25

Thus, the invention provides a method of identifying a binding molecule having selective affinity for a ligand wherein the ligand is a tissue or tumor antigen. The invention also provides a method of
5 identifying a binding molecule having selective affinity for a ligand wherein the ligand is a polypeptide or other macromolecule in a cell lysate.

In addition to ligand populations derived from crude cell preparations or other complex compositions,
10 the ligand populations can also be derived from simple populations of substantially purified molecules. For example, if it is desired to produce a binding molecule selective for any one member of a population of ligands, then each individual member can be combined into a single
15 population and screened simultaneously using the methods of the invention. The molecules can be substantially purified or contain various amounts of other irrelevant species. Additionally, in cases such as this, where a binding molecule is desired which is selective for one or
20 a few members of a population, if the individual ligand species can be produced by recombinant or other biological synthetic mechanisms, then cells can be engineered to express the ligands. The ligands can then be screened in crude cell extracts to identify a binding
25 molecule which is selective for at least one ligand in the population. Such cells can be engineered to synthesize each ligand individually and then cell extracts can be pooled for screening. Alternatively, a cell can be engineered to express multiple ligands so
30 that only one or a few cell extracts are required to be combined and screened.

The ligand populations can be composed of different sizes of either substantially purified

26

molecules or crude cell preparations or other complex
compositions. Generally, simple populations of ligands
contain at least two different ligand species, and can be
composed of any of 3, 4, 5, 6, 7, 8, 9 or 10 different

5 ligands. Moderate ligand populations contain between
about ten and several hundreds of different ligand
species. Complex ligand populations contain about tens
of thousands of different ligand species including, for
example, the number of different molecules within a cell.

10 This number is generally thought to be about 10^5. Even
further, recombinant libraries and combinatorial
libraries can be screened which can be composed of ligand
populations of 10^6, 10^7, 10^8 and as large as greater than
10^{10} different species. Each of these population sizes

15 can be used as a ligand population for which to identify
a binding molecule having selective binding affinity.
The choice of the population size and type will depend on
the need and intended use of the binding molecule. One
skilled in the art will know which size and type of

20 population is suitable for a particular need.

       As stated previously, the ligands which
comprise the above described populations can be
essentially any type of molecule such as a polypeptide,
nucleic acid, carbohydrate, lipid or any organic

25 compound. Therefore, it is understood that molecules
discussed above as binding molecules can also be ligands.
For example, an individual or group of cell surface
receptors such as integrins, growth factor receptors or
cytokine receptors can be used as ligands to screen a

30 random peptide library or combinatorial chemical library
to identify binding molecules that could be used as
agonists or antagonists for these receptors.

To conduct a screen for binding molecules which bind to a ligand or group of ligands, the ligands themselves are detected by an appropriate detection method. The detection method can be direct or indirect

5   and can involve detection of, for example, light emission, radioisotopes, color development, or any method which allows the ligand to be detected. Direct detection can involve methods such as radioactive labeling of ligands using metabolic labeling in an appropriate cell

10   or in vitro labeling using in vitro coupled transcription-translation with appropriate radioactive amino acids, covalent modification with a radioactive substrate using an appropriate enzyme such as a kinase, or chemical modification using radioisotopes such as

15   iodination. Direct methods can also involve fusion of an appropriate detection molecule to the ligand. For example, the ligand can be fused to luciferase and detected by light emission or can be fused to lacZ and detected by appropriate calorimetric detection. In the

20   case where a population of ligands is desired to have biologically derived detection moieties, a library containing ligands fused to such detectable molecules as luciferase and lacZ can be used to screen for binding molecules. Direct detection of a ligand can also be

25   performed by covalent modification of the ligand by incorporating a chemical moiety capable of being detected. For example, biotin can be covalently attached to the ligand and subsequently detected by steptavidin using one of the detection methods discussed above.

30   For indirect detection of ligands, a molecule that is not covalently attached to the ligand can be used for detection. A molecule known to interact with the ligand is used for such indirect detection methods. For example, a known molecule such as an antibody capable of

28

specifically detecting the ligand can be employed. The
known molecule is either itself detected by one of the
methods described above for direct detection of the
ligand or is detected by a secondary molecule specific
5   for the first known molecule. For example, an antibody
specific for a ligand can be detected using a secondary
antibody capable of interacting with the first antibody
specific for the ligand, again using the detection
methods described above for direct detection.

10   In one embodiment, ligands are derived from the
cell surface of tumor cells. Cell surface molecules can
be labeled, for example, with a detectable moiety such as
a radioisotope or biotin. This labeling provides a
source of ligand where the only characteristic that needs
15   to be known is that it is on the surface of a cell. As
described in the Examples, tumor antigen ligands were
prepared by labeling cell surface polypeptides of breast
carcinoma cells with biotin and used to identify Fab
fragment binding molecules selective for the tumor cell
20   surface polypeptides. Ligand populations derived from
other cell or subcellular compartments can similarly be
used in the methods of the invention to obtain a binding
molecule that exhibits selective affinity for at least
one ligand within the initial population. Thus, the
25   methods of the invention are applicable to a large
variety of ligand populations in which selective binding
affinity is required for the therapeutic treatment or
diagnosis of a disease.

     The methods of the invention consist of
30   selectively immobilizing a diverse population of binding
molecules to a solid support. For selective
immobilization, either an inherent characteristic of the
binding molecules which comprise the population is

29

exploited to provide selective immobilization or,
alternatively, the molecules are engineered to contain a
specific characteristic to be used for selective
immobilization. For example, a binding molecule itself

5   may contain a hydrophobic chemical group or domain or may
be fused to a hydrophobic chemical group or domain that
causes the binding molecule to be immobilized to a
hydrophobic solid support such as plastic. In another
example, the solid support can be coated with a chemical

10 moiety or a biomolecule such that it is able to bind to
and selectively immobilize only the binding molecules
that make up the binding molecule population. For
example, the solid support can be coated with a
biomolecule that selectively binds to a domain or

15 sequence that is common to the binding molecules. The
use of such biomolecules as linkers or tethers should be
selected so that they do not interfere with the ligand
binding to the binding molecules. For example, an
antibody against the constant region of an antibody can

20 be used to selectively immobilize antibody binding
molecules. Such an approach was used to immobilize human
Fab fragments with anti-human kappa antibodies as
described in the Examples. Alternatively, antibodies
specific to peptide tags similarly function to

25 selectively immobilize a binding molecule population onto
a solid support.

        One advantage of selective immobilization is
that it provides increased sensitivity and decreased
non-specific binding for detecting specific binding

30 molecules of interest. Solid supports can, for example,
immobilize a high concentration of binding molecules.
Such high concentrations of binding molecules can serve
to increase the ability to detect low affinity
interactions with ligands and to detect ligands in low

30

concentrations. Essentially any solid support is
amenable for use in the methods of the invention. The
support should be chosen to fit a particular need and to
exhibit the capacity to immobilize all, or a
5   statistically representative number of binding molecules
of a population. In addition, solid supports can be made
of porous materials which allow greater densities of
immobilized binding molecules to be achieved.

     Solid supports can also be chosen to increase
10   manipulability of a desired screen while maintaining the
ability to retain the binding molecule population. Such
manipulations can be important for removing unbound
ligand populations and for washing to remove non-specific
interactions. The ease of manipulation can be an
15   advantage when performing high throughput screening of a
large number of binding molecules.

     To perform the methods of the invention, once
the population of binding molecules are immobilized on a
solid support, the immobilized population is
20   simultaneously contacted with a population of two or more
ligands. The population of ligands is incubated with the
selectively immobilized binding molecules under
appropriate conditions for an appropriate period of time
so as to allow binding of the ligands to the binding
25   molecules. A binding molecule having selective affinity
is identified by determining the binding of at least one
ligand within the population to at least one binding
molecule. One skilled in the art will know the
appropriate conditions and can readily determine
30   conditions sufficient to allow interactions between
binding molecules and ligands so as to detect the
selective binding of a binding molecule to a ligand, thus

allowing the identification of a binding molecule having
selective affinity for a ligand.

In addition, the invention provides methods for
determining the sequence of identified binding molecules.
5   For example, if the binding molecules are produced in an
expression library, encoding nucleic acids can be
isolated from selected clones expressing binding
molecules identified in a screen. The encoding nucleic
acids can then be sequenced using methods known to those
10 skilled in the art.

Additionally, the invention provides a method
for characterizing a ligand which is selectively bound by
a binding molecule. Once a binding molecule has been
identified which is selective for a ligand, the ligand
15 can then be isolated by, for example, affinity methods
known to those skilled in the art, and characterized.
This characterization can be beneficial if the ligands
used in the screen are not well characterized.
Characterization of the ligand includes such techniques
20 as determining its apparent molecular weight by gel
electrophoresis. Other methods applicable for
characterizing ligands include, for example, high
performance liquid chromatography (HPLC), mass
spectrometry, or other methods that provide information
25 about the physical, biochemical or functional properties
of the ligand. One skilled in the art will know
appropriate methods for such characterization of ligands.

The invention additionally provides a method
for identifying an antibody having selective affinity for
30 a tumor antigen. The method consists of: a) selectively
immobilizing a diverse population of antibodies to a
solid support; b) simultaneously contacting the diverse

32

population immobilized on the solid support with two or
more tumor antigens; and c) determining at least one
antibody which selectively binds to one or more of the
tumor antigens.

5   The methods of the invention can be practiced
using antibody and tumor cells as the binding molecules
and ligand populations, respectively. The population of
antibody binding molecules can be produced, for example,
in an expression library. Labeling of cell surface
10   molecules of a tumor cell provides a population of
detectable tumor antigen ligands.

           Using the methods of the invention, the
antibody binding molecules are selectively immobilized to
a solid support by, for example, the use of antibodies
15   which are specific to the constant region of antibody
binding molecules or specific to a particular isotype or
antibody subunit. For example, anti-kappa chain
antibodies can be used to immobilize intact antibodies as
well as Fab fragments to a solid support.

20   Antibody binding molecules immobilized to a
solid support can be incubated with tumor antigens under
appropriate conditions so as to allow the tumor antigens
to bind to the antibody binding molecules. The antigens
can be applied as whole cells, cell extracts or as
25   specific cellular fractions. After an appropriate period
of time, unbound tumor antigens are removed from the
solid support and selective binding is detected as
previously described. One skilled in the art will know
what conditions will allow binding of tumor cell ligands
30   to antibodies and can readily determine the conditions
sufficient to allow interactions to allow identification
of antibodies specific for tumor antigens. Tumor

33

antigens are detected to determine one or more antibody
binding molecules which specifically interact with tumor
antigens. The selectivity of an antibody binding
molecule for a tumor antigen can be determined relative
5   to a normal cell which is used as a control. Thus, a
method of the invention provides a means to identify
tumor antigen-specific antibody binding molecules which
can be used for diagnostic or therapeutic purposes.

          Numerous antibody binding molecules selective
10   for tumor specific ligands have been identified by the
methods of the invention. These antibody binding
molecules are described below in the Examples. Moreover,
the nucleotide sequences have been determined for the CDR
regions of these tumor selective antibody binding
15   molecules. Such encoding nucleic acids are similarly
described below in the Examples. Thus, the invention
provides a method of identifying a population of human
antibodies or functional fragments thereof.

          Also provided herein is an isolated binding
20   polypeptide selective for a tumor antigen. The isolated
binding polypeptide is an antibody fragment selected from
the group of antibody fragments consisting of F3, F13,
F14, F15, F19, F21, F22, F23, F26, F30, F31, F32, F33,
F34, F35, F36, F37, F38, F40, F41, F42, F46, F49, F50,
25   F52, F54, F55, F58, F63, F66, F67, F68, F69, F70, F72,
F74, F76, F78, F79, F80, F81, F84, F85, F86, F93, F99,
F104, F111, F112, F118, F126, F129, F130, F132, F133,
F134, F135, F136, F138, F151, F158, F160, F174, F176,
F177, F184, F186, F191, F197, F200, F202, F203, F207,
30   F208, F212, F214, F217, F224, F231, F236, F238, TA50 and
TA73. Such an isolated binding polypeptide selective for
a tumor antigen can be, for example, an F3, F14, F15,

34

F19, F21, F22, F23, F26, F133, TA50 or TA73 antibody
fragment.

A particularly useful isolated binding
polypeptide selective for a tumor antigen can be an
5 antibody fragment having an amino acid sequence including
three particular light chain CDR sequences and three
particular heavy chain CDR sequences. An isolated
antibody fragment selective for a tumor antigen can have,
for example, an amino acid sequence including the three
10 light chain CDR sequences SEQ ID NO:6, SEQ ID NO:28 and
SEQ ID NO:50 and the three heavy chain CDR sequences SEQ
ID NO:72, SEQ ID NO:94 and SEQ ID NO:116 found in F3, or
can have an amino acid sequence in which one or more of
these CDRs are replaced by a substantially similar or
15 unrelated CDR. From the above description, one skilled
in the art understands that an isolated antibody fragment
of the invention can have any sub-combination of the six
F3 CDRs, such as any two, three, four or five F3 CDRs, or
can have a single F3 CDR. An isolated antibody fragment
20 selective for a tumor antigen also can have an amino acid
sequence including the three light chain CDR sequences
SEQ ID NO:8, SEQ ID NO:30 and SEQ ID NO:52 and the three
heavy chain CDR sequences SEQ ID NO:74, SEQ ID NO:96 and
SEQ ID NO:118 found in F14, or can have an amino acid
25 sequence in which one or more of these CDRs are replaced
by a substantially similar or unrelated CDR. From the
above description, one skilled in the art understands
that an isolated antibody fragment of the invention can
have any sub-combination of the six F14 CDRs, such as any
30 two, three, four or five F14 CDRs, or can have a single
F14 CDR.

In another embodiment, an isolated antibody
fragment selective for a tumor antigen can have an amino

acid sequence including the three light chain CDR
sequences SEQ ID NO:10, SEQ ID NO:32 and SEQ ID NO:54 and
the three heavy chain CDR sequences SEQ ID NO:76, SEQ ID
NO:98 and SEQ ID NO:120 found in F15, or can have an

5   amino acid sequence in which one or more of these CDRs
are replaced by a substantially similar or unrelated CDR.
From the above description, one skilled in the art
understands that an isolated antibody fragment of the
invention can have any sub-combination of the six F15

10   CDRs, such as any two, three, four or five F15 CDRs, or
can have a single F15 CDR. In another embodiment, an
isolated antibody fragment selective for a tumor antigen
can have an amino acid sequence including the three light
chain CDR sequences SEQ ID NO:12, SEQ ID NO:34 and SEQ ID

15   NO:56 and the three heavy chain CDR sequences SEQ ID
NO:78, SEQ ID NO:100 and SEQ ID NO:122 found in F19, or
can have an amino acid sequence in which one or more of
these CDRs are replaced by a substantially similar or
unrelated CDR. From the above description, one skilled

20   in the art understands that an isolated antibody fragment
of the invention can have any sub-combination of the six
F19 CDRs, such as any two, three, four or five F19 CDRs,
or can have a single F19 CDR.

25   In another embodiment, an isolated antibody
fragment selective for a tumor antigen can have an amino
acid sequence including the three light chain CDR
sequences SEQ ID NO:14, SEQ ID NO:36 and SEQ ID NO:58 and
the three heavy chain CDR sequences SEQ ID NO:80, SEQ ID
NO:102 and SEQ ID NO:124 found in F21, or can have an

30   amino acid sequence in which one or more of these CDRs
are replaced by a substantially similar or unrelated CDR.
From the above description, one skilled in the art
understands that an isolated antibody fragment of the
invention can have any sub-combination of the six F21

36

CDRs, such as any two, three, four or five F21 CDRs, or can have a single F21 CDR. In another embodiment, an isolated antibody fragment selective for a tumor antigen can have an amino acid sequence including the three light

5   chain CDR sequences SEQ ID NO:16, SEQ ID NO:38 and SEQ ID NO:60 and the three heavy chain CDR sequences SEQ ID NO:82, SEQ ID NO:104 and SEQ ID NO:126 found in F22, or can have an amino acid sequence in which one or more of these CDRs are replaced by a substantially similar or

10 unrelated CDR. From the above description, one skilled in the art understands that an isolated antibody fragment of the invention can have any sub-combination of the six F22 CDRs, such as any two, three, four or five F22 CDRs, or can have a single F22 CDR.

15   In another embodiment, an isolated antibody fragment selective for a tumor antigen can have an amino acid sequence including the three light chain CDR sequences SEQ ID NO:18, SEQ ID NO:40 and SEQ ID NO:62 and the three heavy chain CDR sequences SEQ ID NO:84, SEQ ID

20 NO:106 and SEQ ID NO:128 found in F23, or can have an amino acid sequence in which one or more of these CDRs are replaced by a substantially similar or unrelated CDR. From the above description, one skilled in the art understands that an isolated antibody fragment of the

25 invention can have any sub-combination of the six F23 CDRs, such as any two, three, four or five F23 CDRs, or can have a single F23 CDR. In another embodiment, an isolated antibody fragment selective for a tumor antigen can have an amino acid sequence including and the three

30 light chain CDR sequences SEQ ID NO:20, SEQ ID NO:42 and SEQ ID NO:64 and the three heavy chain CDR sequences SEQ ID NO:86, SEQ ID NO:108 and SEQ ID NO:130 found in F26, or can have an amino acid sequence in which one or more of these CDRs are replaced by a substantially similar or

37

unrelated CDR. From the above description, one skilled
in the art understands that an isolated antibody fragment
of the invention can have any sub-combination of the six
F26 CDRs, such as any two, three, four or five F26 CDRs,
5   or can have a single F26 CDR.

In another embodiment, an isolated antibody
fragment selective for a tumor antigen can have an amino
acid sequence including and the three light chain CDR
sequences SEQ ID NO:22, SEQ ID NO:44 and SEQ ID NO:66 and
10 the three heavy chain CDR sequences SEQ ID NO:88, SEQ ID
NO:110 and SEQ ID NO:132 found in F133, or can have an
amino acid sequence in which one or more of these CDRs
are replaced by a substantially similar or unrelated CDR.
From the above description, one skilled in the art
15 understands that an isolated antibody fragment of the
invention can have any sub-combination of the six F133
CDRs, such as any two, three, four or five F133 CDRs, or
can have a single F133 CDR.

In another embodiment, an isolated antibody
20 fragment selective for a tumor antigen can have an amino
acid sequence including and the three light chain CDR
sequences SEQ ID NO:24, SEQ ID NO:46 and SEQ ID NO:68 and
the three heavy chain CDR sequences SEQ ID NO:90, SEQ ID
NO:112 and SEQ ID NO:134 found in TA50, or can have an
25 amino acid sequence in which one or more of these CDRs
are replaced by a substantially similar or unrelated CDR.
From the above description, one skilled in the art
understands that an isolated antibody fragment of the
invention can have any sub-combination of the six TA50
30 CDRs, such as any two, three, four or five TA50 CDRs, or
can have a single TA50 CDR.

38

In still another embodiment, an isolated antibody fragment selective for a tumor antigen can have an amino acid sequence including and the three light chain CDR sequences SEQ ID NO:26, SEQ ID NO:48 and SEQ ID
5   NO:70 and the three heavy chain CDR sequences SEQ ID NO:92, SEQ ID NO:114 and SEQ ID NO:136 found in TA73, or can have an amino acid sequence in which one or more of these CDRs are replaced by a substantially similar or unrelated CDR. From the above description, one skilled
10 in the art understands that an isolated antibody fragment of the invention can have any sub-combination of the six TA73 CDRs, such as any two, three, four or five TA73 CDRs, or can have a single TA73 CDR.

Additionally provided is a CDR or functional
15 fragment thereof of an antibody selective for a tumor antigen, where the CDR is derived from an antibody fragment selected from the group consisting of F3, F13, F14, F15, F19, F21, F22, F23, F26, F30, F31, F32, F33, F34, F35, F36, F37, F38, F40, F41, F42, F46, F49, F50,
20 F52, F54, F55, F58, F63, F66, F67, F68, F69, F70, F72, F74, F76, F78, F79, F80, F81, F84, F85, F86, F93, F99, F104, F111, F112, F118, F126, F129, F130, F132, F133, F134, F135, F136, F138, F151, F158, F160, F174, F176, F177, F184, F186, F191, F197, F200, F202, F203, F207,
25 F208, F212, F214, F217, F224, F231, F236, F238, TA50 and TA73. Such a CDR can have, for example, an amino acid sequence that is substantially similar or identical to the CDR-encoding portion of an amino acid sequence encoding one of these antibody fragments.

30   The present invention also provides an isolated nucleic acid molecule encoding an antibody fragment of the invention. Such a nucleic acid molecule includes a nucleotide sequence encoding substantially the amino acid

39

sequence referenced as SEQ ID NO:6, SEQ ID NO:8, SEQ ID
NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID
NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID
NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID
5   NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID
NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID
NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID
NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID
NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID
10 NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID
NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID
NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID
NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ
ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112,
15 SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID
NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ
ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134,
or SEQ ID NO:136.

A nucleic acid molecule encoding an antibody
20 fragment can include, for example, the nucleotide
sequence referenced as SEQ ID NO:5, SEQ ID NO:7, SEQ ID
NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID
NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID
NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID
25 NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID
NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID
NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID
NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID
NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID
30 NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID
NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID
NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID
NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID
NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ

ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119,
SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID
NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, or
SEQ ID NO:135.

5   In one embodiment, a CDR or functional fragment
thereof of an antibody selective for a tumor antigen can
be derived from an F3, F14, F15, F19, F21, F22, F23, F26,
F133, TA50 or TA73 antibody fragment. Such a CDR can
include substantially the amino acid sequence referenced
10 as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12,
SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20,
SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28,
SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36,
SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44,
15 SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52,
SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60,
SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68,
SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76,
SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84,
20 SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92,
SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100,
SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID
NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ
ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122,
25 SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID
NO:130, SEQ ID NO:132, SEQ ID NO:134, or SEQ ID NO:136.

        The present invention also provides an isolated
nucleic acid molecule encoding a CDR of the invention.
Such a nucleic acid molecule includes a nucleotide
30 sequence encoding substantially the amino acid sequence
referenced as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ
ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ
ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ

41

ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ

ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ

ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ

ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ

5 ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ

ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ

ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ

ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ

ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ

10 ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106,

SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID

NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ

ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128,

SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, or SEQ ID

15 NO:136.

A nucleic acid molecule encoding a CDR can

include, for example, the nucleotide sequence referenced

as SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11,

SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19,

20 SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27,

SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35,

SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43,

SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51,

SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59,

25 SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67,

SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75,

SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83,

SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91,

SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99,

30 SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID

NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ

ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121,

SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID

NO:129, SEQ ID NO:131, SEQ ID NO:133, or SEQ ID NO:135.

42

It is understood that modifications which do
not substantially affect the activity of the various
embodiments of this invention are also included within
the definition of the invention provided herein.
5   Accordingly, the following examples are intended to
illustrate but not limit the present invention.

                          EXAMPLE I
       Preparation of Solid Supports Selectively Immobilizing
                   Human Fab Fragment Populations

10   This example describes a method for preparing a
filter for selectively immobilizing a population of
binding molecules.

            The population of binding molecules selectively
immobilized in this example were recombinantly expressed
15 human Fab fragments. In this case, the human BR96 Fab
fragment, which is a chimeric antibody reactive with a
tumor-associated cell surface antigen, was used to
determine conditions for binding human Fab fragments.
The BR96 antibody is reactive with a Lewis Y-related
20 carbohydrate (LeY antigen) predominantly associated with
lamp-1, an integral membrane protein normally localized
in lysosomes. The population of human Fab fragments was
immobilized on a solid support by coating nitrocellulose
filters with anti-human kappa antibody. The anti-human
25 kappa antibody was used as an agent for attachment to the
filters since they are specific for the antibody
population and can therefore be used to selectively
immobilize or "capture" the population onto the filters.

            Initially, conditions were determined for
30 optimizing the amount of antibody that could be
selectively immobilized on nitrocellulose filters. A
variety of anti-human immunoglobulin reagents were tested

43

as reagents for selective immobilization of human Fab
fragments. Virtually all anti-human immunoglobulin
reagents tested displayed enhanced signals compared to
untreated nitrocellulose filters and it was determined

5   that an affinity-purified polyclonal goat anti-human
kappa antibody was most efficient at selectively
immobilizing the BR96 Fab fragment. The non-specific
signal generated by this approach was determined by
overlaying filters both on areas not infected with phage

10 and on phage plaques expressing an irrelevant antibody.
The assay background was low and no difference was
observed in the signal generated by either of these
controls.

        Using the polyclonal anti-human kappa antibody

15 for selective immobilization, the concentration of anti-
human kappa antibody for maximal binding of BR96 Fab
fragment was determined. Briefly, molten 0.7% agar
containing 1 mM isopropyl-β-D-thiogalactopyranoside
(IPTG) and the $supE$ amber suppressor strain XL1-Blue

20 (Stratagene, San Diego, CA) was overlaid on hardened
bottom 1.5% agar Luria Broth medium and incubated at 37°C
for 3 hours. A 3 μl aliquot of phage stock (~10^{12}
phage/ml) was spotted directly on the bacterial lawn and
allowed to seep into the agar for 15 minutes at 25°C.

25 The plate was then transferred to 37°C for 3-4 hours at
which time large plaques were visible. Plaques were
overlaid with 1 cm^2 nitrocellulose filters. The filters
were removed and rinsed four times with 1.5 mM KH_2PO_4, 8
mM Na_2HPO_4, 2.7 mM KCl, 137 mM NaCl, pH 7.5 (PBS). To

30 detect the selective immobilization of functional BR96
Fab, the filters were placed in PBS containing 0.772
μg/ml horseradish peroxidase-conjugated Lewis Y antigen,
5% nonfat powdered milk, 0.2% Tween 20, 0.01% antifoam A
emulsion and 0.01% thimerosal and incubated for 2 hours

35 at 4°C. The filters were washed five times with PBS and
placed in 50 mM citric acid, 100 mM Na_2HPO_4, pH 5,

44

containing 1 mg/ml o-phenylenediamine dihydrochloride and
0.003% hydrogen peroxide. The reaction was terminated by
the addition of H_2SO_4 to a final concentration of 0.36 M,
an aliquot was removed, and the OD_{490} was measured in a
5   spectrophotometer.

       As shown in Figure 1A, increasing
concentrations of anti-human kappa antibody up to
approximately 10 μg/ml retained greater quantities of
BR96 Fab fragment. Filters coated with more than 10
10   μg/ml anti-human kappa antibody generated an enhanced,
but sub-optimal signal while coating with less than 1
μg/ml antibody did not amplify the signal appreciably.
Untreated filters and filters coated with low
concentrations of anti-human kappa antibody (less than
15   0.2 μg/ml) did not generate an assay signal above
background (A_{490} ~0.05). Therefore, based on the signal
obtained from filters coated with 10 μg/ml anti-human
kappa antibody (A_{490} ~0.45), the selective immobilization
filter lift technique amplified the signal by at least
20   nine-fold.

       The amount of functional antibody Fab fragment
expressed and selectively immobilized by the coated
filters was assessed at various times from 0 to 8 hours
at 25oC. As shown in Figure 1B, the signal increased
25   from 0 to 4 hours, at which point additional transfer
time did not increase the signal appreciably.

       Based on these results, nitrocellulose filters
were incubated 3 hours in a solution containing 10 μg/ml
of goat anti-human kappa antibody in PBS. To block
30   unreacted sites on the nitrocellulose, the nitrocellulose
filters were incubated 2 hours in a solution containing
1% bovine serum albumin (BSA) in PBS.

45

        Filters prepared by this method were used in
the studies described below.

                        EXAMPLE II
    Immobilization of Human Fab Fragments on Solid Supports
5   Enhanced Sensitivity for Detecting Human Fab Fragments

        This example demonstrates that immobilization
of binding molecule populations increases the sensitivity
of detecting specific binding events.

        A phage library expressing BR96-reactive Fab
10 (240H2) was plated on E. coli bacterial lawns and grown
    at 37°C for 8 hours. Control filter lifts were generated
    by applying nitrocellulose filters to the bacterial lawns
    and incubating at 22°C for 4 hours. These control
    nitrocellulose filters were removed. Control filter
15 lifts, therefore, have not selectively immobilized the
    binding molecules expressed in phage plaques but instead
    have directly immobilized all secreted polypeptides and
    polypeptides released by lysis, including the binding
    molecules. To selectively immobilize human Fab
20 fragments, nitrocellulose filters coated with goat anti-
    human kappa antibody, prepared as in Example I, were
    applied to the bacterial lawns and incubated at 22°C for
    4 hours. These selectively immobilized filter lifts were
    removed. Subsequent steps were carried out in parallel
25 with control filter lifts and selectively immobilized
    filter lifts. Filter lifts were blocked in 1% BSA and
    incubated in horse radish peroxidase-conjugated BR96
    antigen (LeY antigen). Filters were developed by
    enhanced chemiluminescence (ECL) using procedures
30 described by the manufacturer (Amersham; Arlington
    Heights IL) and exposed to film for 30 seconds. The
    results were independent of the order the filters were
    applied to the bacterial lawn.

46

As shown in Figure 2, human Fab fragments were
readily detected on selectively immobilized filter lifts
(designated capture lift in Figure 2) whereas control
filter lifts (designated normal lift in Figure 2) had
5   very low levels of detectable human Fab fragments.
Selectively immobilized filter lifts had much stronger
signals for human antibodies that recognize the tumor
antigen BR96 when compared to control filter lifts where
the nitrocellulose filters are applied directly to the
10   bacterial lawn. Thus, using a selectively immobilized
filter lift to increase the concentration of the specific
binding molecule of interest, in this case human Fab
fragments, gave greater sensitivity for detecting the
binding molecule of interest.

15   These results show that selectively immobilized
filter lifts have increased sensitivity and decreased
background binding compared to control filter lifts where
binding molecules are attached directly to the filter.

EXAMPLE III
20   Preparation and Extraction of Biotinylated Cell Surface
Antigens

This example describes a method for labeling
cell surface tumor antigens. This example also describes
the preparation of cell surface tumor antigen extracts
25   that give low background binding when used for detecting
selectively immobilized antibodies.

H3396 cells were grown in monolayers and
chilled on ice for subsequent manipulations at 4°C.
Cells were rinsed three times with PBS. After 30
30   minutes, cells were incubated for 2 hours with 1.5 mg/ml
sulfosuccinimidyl-6-(biotinamido)hexanoate (Pierce) in
PBS. Cells were rinsed once with PBS containing 50 mM
glycine followed by two more rinses with PBS. Cells were

47

harvested by scraping in PBS containing 100 μM [4-2-
aminoethyl)-benzenesulfonylfluoride, HCl], 1 μM pepstatin
A and 10 μM leupeptin hemisulfate. The protein
concentration was determined, Triton X-100 was added to a
5   final concentration of 10:1 (w:w) and the suspension was
incubated overnight at 4°C on a rotating wheel.
Particulate material was removed by centrifugation at
11,000 x g for 30 minutes at 4°C followed by
ultracentrifugation at 540,000 x g for 7 minutes 4°C.
10   The supernatant contains solubilized biotinylated cell
surface antigens.

        These results demonstrate that cell surface
polypeptides can be labeled with biotin for use in
screening methods. These results also demonstrate that
15   extracts devoid of interfering particulate matter,
including microsomes, can be prepared for use in probing
selectively immobilized filter lifts with low background
binding.

                        EXAMPLE IV
20   Decreased Background of Selectively Immobilized Filter
              Lifts Relative to Control Filter Lifts

        This example demonstrates that selectively
immobilized filter lifts provide decreased background
binding of non-specific bacterial proteins relative to
25   control filter lifts.

        Phage expressing BR96-reactive Fab (240H2) were
mixed with an irrelevant phage stock (IX64), which does
not express any Fab, and plated on an E. coli bacterial
lawn and grown for 8 hours at 37°C. Four replicate
30   filter lifts (two control filter lifts and two
selectively immobilized filter lifts) were incubated
sequentially with the bacterial lawns for 4 hours at
22°C. Two control filter lifts (A and B) and two

48

selectively immobilized filter lifts (C and D) were
incubated with various reagents. To localize all plaques
present on the bacterial lawn, filter lift A was
incubated with rabbit anti-fd bacteriophage antibody in
5   1% BSA in PBS containing 0.1% sodium azide, washed three
times in PBS, and subsequently incubated in alkaline
phosphatase goat anti-rabbit antibody. Filters were
washed six times with PBS and developed by incubation in
alkaline phosphatase calorimetric reagent containing 0.1
10   M Tris, pH 9.5, 0.4 mM 2,2'-di-p-nitrophenyl-5,5'-
diphenyl-3,3'-(3,3'-dimethoxy-4,4'-
diphenylene)ditetrazolium chloride and 0.38 mM 5-bromo-4-
chloro-3-indoxyl phosphate mono-(p-toluidinium) salt.

Filter lift C was incubated with horse radish peroxidase-
15   conjugated Lewis Y antigen (BR96 antigen). Filter lift C
was developed using ECL and exposed to film for 30
seconds.

To investigate the ability of selectively
immobilized filter lifts to discriminate ligands in a
20   cell extract, H3396 cells were biotinylated and membrane
extracts were prepared as described in Example III.
Filter lifts B and D were incubated for 2 hours at 4°C
with biotinylated cell surface antigens, prepared as
described in Example III and diluted to 40 μg/ml tumor
25   cell extract protein in 1% BSA, 1% Triton X-100, 0.145%
sodium dodecyl sulfate and 0.1% sodium azide in PBS.
Filter lifts B and D were washed six times with 0.1%
Tween 20 in PBS and subsequently incubated with alkaline
phosphatase-conjugated streptavidin. Plaques were
30   visualized after developing in alkaline phosphatase
calorimetric reagent.

As shown in Figure 3, all plaques were
visualized with anti-fd bacteriophage antibody on filter
lift A. Similarly, all plaques were visible when control
35   lift B was probed with biotinylated cell surface

49

antigens, even though plaques derived from IX64 do not
express Fab fragments. Therefore, there appears to be
significant background contributed by bacterial proteins
released by phage lysis. Numerous attempts to reduce the
5   non-specific binding of tumor cell extract were made,
including: (1) utilization of a variety of detergents for
the solubilization of the tumor cell membranes, (2) use
of more stringent wash parameters with the filters, (3)
evaluation of a wide range of blocking reagents, and (4)
10   the use of different types of matrices to capture the
phage-expressed Fab. All of these approaches either
failed to reduce the background or obliterated the signal
entirely.

In contrast to control filters containing
15   directly bound binding molecules and non-specific
bacterial proteins, probing selectively immobilized
filter lifts with purified BR96 antigen (Figure 3, filter
lift C) or with a cell membrane lysate containing BR96
antigen as well as other biotinylated cell surface
20   antigens (filter lift D) gave similar patterns of plaque
detection. The use of crude, cell membrane lysate as
source of BR96 antigen resulted in non-specific binding
to all plaques, including those not expressing BR96-
specific Fab fragments, on control filter lifts (filter
25   lift B) whereas the same extract could be used to detect
BR96-specific Fab fragments on selectively immobilized
filter lifts (filter lift D). Therefore, selectively
immobilized filter lifts had significantly lower
background binding to bacterial proteins than control
30   filter lifts.

These results indicate that selective
immobilization of binding molecules to nitrocellulose
filters produces decreased background binding to
bacterial proteins. Therefore, capturing the binding
35   molecule of interest by selectively immobilizing binding

50

molecules on filters increased the concentration of the
binding molecule by selectively capturing the binding
molecule in the bacterial plaque lysate. In addition,
background was decreased by reducing the binding of non-
5   specific bacterial proteins. Thus, the selectively
immobilized filter lift method provides enhanced
sensitivity and decreased background when screening
binding molecules with a population of ligands.

EXAMPLE V
10   Identification of Tumor-Specific Fab Fragments Identified
by Selective Immobilization on a Solid Support

This example demonstrates the identification of
clones expressing tumor-specific Fab fragments.

Fab fragment libraries were generated using
15   breast and colon tumor tissue. A large number of Fab
fragments were identified with specificity for cell
surface tumor antigens.

Three libraries expressing human Fab fragments,
designated A2, A3 and A4, were generated from three
20   different tumor sources. The A2 library was constructed
from tumor-draining lymph node tissue obtained from a
breast cancer patient. The A3 library was constructed
from human colon tumor tissue. The A4 library was
constructed from prostate tumor tissue. The libraries
25   were constructed from B cells present in the tumor tissue
samples. Briefly, RNA was extracted from tumor tissue
using TRIZOL reagent (Gibco-BRL, Gaithersburg, MD).
Following synthesis of cDNA from the RNA, the IgG heavy
and kappa light chains were amplified by touchdown
30   polymerase chain reaction (PCR). Single-stranded DNA was
isolated and cloned into two vectors, IX104 and IX203.
The IX104 vector contains the amber stop codon between

51

the immunoglobulin heavy chain and the pVIII protein.
The IX203 vector has the amber stop codon removed.

        The human Fab libraries were selectively
immobilized on nitrocellulose filters coated with
5   anti-human kappa antibody (prepared as in Example I).
Outlined in Table II are the results of three independent
experiments screening the human Fab libraries in either
the IX104 or IX203 vector against biotinylated cell
surface polypeptides from the H3396 cell line (prepared
10 as described in Example III). Using the selectively
immobilized filter lift technique, the frequency of
positive clones varied in the three independent
experiments (see Table II). As defined in these
experiments, the positive clones (designated capture
15 lift-positive clones in Table II) were identified in an
initial screen, picked, replated and verified in a second
selectively immobilized filter lift screen.

Table II. SUMMARY OF FAB LIBRARY SCREEN WITH TUMOR CELLS

Library	Vector	Random Clones Screened	Capture Lift- Positive Clones	Frequency	Titrate on Fixed H3396 Monolayers	Fibroblast -negative[1]
A2	IX104	50,600	60	0.12%	25	2+
A3	IX104	139,400	70	0.05%	37	9+
A3	IX203	55,520	51	0.09%	5	0
A4	IX203	13,000	22	0.17%	6	2

[1]unreactive at Fab fragment concentrations ≤ 15 $\mu g/ml$

25   Positive clones identified by the selectively
immobilized filter lift technique were isolated and used
as a source to produce greater quantities of soluble Fab
for further characterization. Briefly, XL1-Blue bacteria
were grown in 2X YT medium at 37°C until the cultures
30 reached a density of 0.9 to 1.2 OD_{600}. Following the

52

addition of IPTG to a final concentration of 1 mM, the
culture was infected with 10 μl of phage from a high
titer stock (>10^{10} plaque forming units/ml) and incubated
1 hour at 37°C. The cultures were shifted to 25°C and
5   grown for an additional 14-16 hours. The cells were
pelleted at 6000 x g for 10 minutes, resuspended in 10 ml
of 50 mM Tris, pH 8.0 containing 100 μM (4-(2-
aminoethyl)-benzenesulfonylfluoride, HCl), 1 μM pepstatin
A, and 10 μM leupeptin hemisulfate, and were broken by
10   sonic oscillation for 1 minute at 15% power output with a
Branson Sonifier 450 equipped with a microtip. Following
sonication, 1000 U DNaseI in 50 mM Tris, pH 8.0, was
added and the lysate was incubated for 30 minutes at
25°C. The lysate was centrifuged at 12,000 x g for 30
15   minutes at 4°C and the supernatant was removed and used
for purification of the Fab fragment.

     The Fab fragment was purified using an affinity
column constructed by coupling 20 mg of goat anti-human
IgG Fab onto a 1 ml HiTrap NHS-activated column
20   (Pharmacia, Piscataway, NJ). Tween 20 was added to the
bacterial cell lysate to a final concentration of 0.05%
and the lysate was filtered through a 0.45 μm filter and
applied to the affinity column. The unbound fraction was
collected and applied a second time. The column was
25   washed with buffer containing 50 mM Tris, pH 8.0, 0.5 M
NaCl and 0.05% Tween 20 followed by 50 mM Tris, pH 8.0
buffer. The Fab was eluted with 100 mM glycine-HCl, pH
2.3, containing 0.5 M NaCl into one tenth volume 1 M
Tris, pH 8.0. Fractions containing the Fab fragment were
30   applied to a PD-10 Sephadex G25-M column (Pharmacia)
which had been equilibrated in PBS.

     To further characterize purified Fab fragments,
H3396 cell monolayers were fixed with 2% paraformaldehyde
in PBS for 15 minutes at 25°C. The paraformaldehyde was
35   aspirated and cells were washed twice with PBS. Fixed

cells were incubated in blocking buffer containing 1% BSA
in PBS and Fab fragments were diluted to appropriate
concentrations in blocking buffer and incubated with the
fixed cells for 2 hours at 25ºC. The samples were
5   aspirated, the fixed cells washed three times with PBS
and the cells were incubated with 0.5 μg/ml alkaline
phosphatase-conjugated goat anti-human kappa light chain
antibody for 1 hour at 25ºC. The fixed cells were washed
four times with PBS and developed with alkaline
10   phosphatase calorimetric reagent.

Approximately 40-50% of the Fab fragments
identified by selective immobilization on filter lifts
generated a titratable signal on fixed monolayers of
H3396 tumor cells, the cell line used to generate the
15   biotinylated cell membrane lysate (see Table II). Some
of the approximately 50% of Fab fragments which were not
titrated on fixed monolayers are likely dependent on
antigenic epitopes which were disrupted by
paraformaldehyde treatment. Different techniques which
20   do not use paraformaldehyde treatment can be used to
characterize these remaining Fab fragment clones.

Positive Fab fragment clones isolated by
selective immobilization on a solid support, designated
capture lift-positive clones in Table II, were also
25   tested for reactivity of the cloned Fab fragments against
GM05658 normal human fibroblast cells. In one experiment
where 50,600 original clones were screened, more than 2
clones did not react with fibroblast cells and were
classified as fibroblast-negative. In a second
30   experiment where 139,400 original clones were screened,
more than 9 clones were identified as fibroblast-negative
clones. Thus, selective immobilization allowed the
identification of human Fab fragments which specifically
bind to tumor cells but not normal fibroblasts.

54

Using the selective immobilization filter lift
technique, 81 clones expressing Fab fragments were
identified as shown in Table III.

DNA was isolated from Fab clones, and DNA
5   fragments corresponding to light chain CDR regions were
sequenced using the IX104 specific primer
GCCAGTTCCAGATTTCAACTG (SEQ ID NO:1) or the IX203 specific
primer CTCTGTGACACTCTCCTGGGA (SEQ ID NO:2). DNA
fragments corresponding to heavy chain CDR regions were
10   sequenced using primer GTAGTCCTTGACCAGGCA (SEQ ID NO:3)
or AAGACCGATGGGCCCTTGGTGGAGGC (SEQ ID NO:4). Table IV
shows the amino acid sequences of three CDR regions found
in the light chain of the cloned Fab fragments. Table V
shows the nucleotide sequences of the corresponding CDR
15   regions of the light chain of cloned Fab fragments.
Table VI shows the amino acid sequences of three CDR

55

Table III. Tumor-specific Human Fab Clones Identified by the Selective Immobilization Filter Lift Technique

clone	library	family
F3	A3.104	VH3
F13	A3.104	VH4
F14	A3.104	VH3
F15	A3.104	VH3, VK3
F19	A3.104	VH4
F21	A3.104	VH1
F22	A3.104	VH6
F23	A3.104	VH3, VK1
F26	A3.104	VH3
F30	A2.104	
F31	A2.104	
F32	A2.104	
F33	A2.104	
F34	A3.104	
F35	A3.104	
F36	A3.104	
F37	A3.104	
F38	A3.104	
F40	A2.104	
F41	A2.104	
F42	A2.104	
F46	A2.104	
F49	A2.104	
F50	A2.104	
F52	A2.104	
F54	A2.104	
F55	A2.104	
F58	A2.104	
F63	A3.104	
F66	A3.104	
F67	A3.104	
F68	A3.104	
F69	A3.104	
F70	A3.104	
F72	A3.104	
F74	A3.104	
F76	A3.104	
F78	A3.104	
F79	A3.104	
F80	A3.104	
F81	A3.104	
F84	A3.104	
F85	A3.104	
F86	A3.104	VH3, VK1
F93	A3.203	

5

10

15

20

25

30

35

40

45

56

TABLE III. Tumor-specific Human Fab Clones Identified by the Selective Immobilization Filter Lift Technique

clone	library	family
F99	A3.104	
F104	A3.104	
F111	A3.104	
F112	A3.104	
F118	A3.104	
F126	A3.203	
F129	A3.203	
F130	A3.203	
F132	A3.104	VH3, VK1
F133	A3.104	VH3, VK1
F134	A3.104	VH3, VK1
F135	A3.104	VH3, VK1
F136	A3.104	
F138	A3.203	
F151	A3.203	
F158	A3.203	
F160	A3.203	
F174	A3.203	
F176	A3.203	
F177	A4.203	
F184	A4.203	
F186	A4.203	
F191	A4.203	
F197	A4.203	
F200	A4.203	
F202	A4.203	
F203	A4.203	
F207	A4.203	
F208	A4.203	
F212	A4.203	
F214	A4.203	
F217	A4.203	
F224	A4.203	
F231	A4.203	
F236	A4.203	
F238	A4.203	
TA50	A3.104	VH5, VK3
TA73	A3.104	VH1, VK1

regions found in the heavy chain of the cloned Fab
fragments.   Table VII shows the nucleotide sequences of
the corresponding CDR regions of the heavy chain of
cloned Fab fragments. Each of the Fab clones identified
by selective immobilization on filters contains
nucleotide sequence encoding unique amino acid sequence.

57

Table IV.   Amino Acid Sequences of CDR Regions in the Light Chains of Fab Fragments Isolated by the Selective Immobilization Filter Lift Technique

Light Chain CDR 1
Clone

Clone	Sequence												SEQ ID
F3	Q	A	S	H	S	V	F	N	L	V	G		SEQ ID NO:6
F14	R	D	H	S	N	V	L	A	A	A	S	W A	SEQ ID NO:8
F15	R	A	S	Q	R	V	S	S	R	F	V	A	SEQ ID NO:10
F19	R	G	L	V	R	T	L	N	N	H	L	N	SEQ ID NO:12
F21	R	A	S	Q	S	V	S	S	T	N	L	T	SEQ ID NO:14
F22	R	A	K	Q	N	I	G	R	W	L	A		SEQ ID NO:16
F23	R	A	S	Q	D	I	N	T	Y	L	A		SEQ ID NO:18
F26	R	A	V	Q	R	I	K	Q	N	F	G	N	SEQ ID NO:20
F133	R	A	S	Q	D	I	N	T	Y	L	A		SEQ ID NO:22
TA50	R	A	S	Q	T	V	R	S	S	Y	L	A	SEQ ID NO:24
TA73	Q	A	N	E	D	I	G	H	R	L	N		SEQ ID NO:26

Light Chain CDR 2
Clone

Clone	Sequence							SEQ ID
F3	K	S	S	N	F	T	K	SEQ ID NO:28
F14	G	A	S	T	R	A	T	SEQ ID NO:30
F15	G	A	S	S	R	A	S	SEQ ID NO:32
F19	A	A	S	R	L	Q	S	SEQ ID NO:34
F21	A	A	S	T	L	Q	T	SEQ ID NO:36
F22	R	A	S	S	L	E	S	SEQ ID NO:38
F23	R	A	S	T	L	D	I	SEQ ID NO:40
F26	K	A	S	R	L	E	N	SEQ ID NO:42
F133	R	A	S	T	L	D	I	SEQ ID NO:44
TA50	G	A	S	I	R	A	P	SEQ ID NO:46
TA73	R	S	S	N	L	K	K	SEQ ID NO:48

Light Chain CDR 3
Clone

Clone	Sequence										SEQ ID
F3	Q	Q	Y	S	S	T	F	G	T		SEQ ID NO:50
F14	Q	H	Y	K	S	A	P	P	R	H T	SEQ ID NO:52
F15	Q	Q	S	H	R	T	P	Y	T		SEQ ID NO:54
F19	Q	Q	S	S	V	S	R	T			SEQ ID NO:56
F21	Q	H	A	Y	A	F	P	R	T		SEQ ID NO:58
F22	Q	H	Y	N	T	N	S	P	L	T	SEQ ID NO:60
F23	Q	Q	Y	S	R	Y	P	L	T		SEQ ID NO:62
F26	Q	L	Y	D	S	H	S	A	M	Y T	SEQ ID NO:64
F133	Q	Q	Y	S	R	Y	P	L	T		SEQ ID NO:66
TA50	Q	Q	Y	G	R	S	P	R	A		SEQ ID NO:68
TA73	L	Q	D	F	N	Y	P	R	T		SEQ ID NO:70

58

Table V. Nucleotide Sequences of CDR Regions in the Light Chains of Fab Fragments Isolated by the Selective Immobilization Filter Lift Technique

Light Chain CDR 1
Clone
F3	CAG	GCC	AGT	CAC	AGT	GTT	TTT	AAC
	TTG	GTT	GGC					SEQ ID NO:5
F14	AGG	GAC	CAT	TCA	AAC	GTG	TTA	GCA
	GCA	GCT	TCA	TGG	GCC			SEQ ID NO:7
F15	AGG	GCC	AGT	CAG	CGT	GTT	AGC	AGC
	AGA	TTC	GTA	GCT				SEQ ID NO:9
F19	CGG	GGG	CTA	GTC	AGG	ACA	TTA	AAC
	AAC	CAT	CTA	AAT				SEQ ID NO:11
F21	AGG	GCC	AGT	CAG	AGT	GTT	AGC	AGC
	ACT	AAC	TTA	ACT				SEQ ID NO:13
F22	CGG	GCC	AAG	CAG	AAT	ATT	GGT	CGG
	TGG	TTG	GCT					SEQ ID NO:15
F23	CGG	GCC	AGT	CAG	GAC	ATT	AAC	ACT
	TAT	TTA	GCC					SEQ ID NO:17
F26	CGG	GCA	GTT	CAA	CGC	ATT	AAA	CAA
	AAT	TTT	GGG	AAT				SEQ ID NO:19
F133	CGG	GCC	AGT	CAG	GAC	ATT	AAC	ACT
	TAT	TTA	GCC					SEQ ID NO:21
TA50	AGG	GCC	AGT	CAG	ACT	GTT	AGG	TCC
	AGC	TAC	TTA	GCC				SEQ ID NO:23
TA73	CAG	GCG	AAT	GAG	GAC	ATT	GGC	CAC
	CGT	TTA	AAT					SEQ ID NO:25

Light Chain CDR 2
Clone
F3	AAG	TCA	TCT	AAC	TTT	ACA	AAG	SEQ ID NO:27
F14	GGT	GCA	TCC	ACC	AGG	GCC	ACC	SEQ ID NO:29
F15	GGT	GCA	TCC	AGC	AGG	GCT	TCT	SEQ ID NO:31
F19	GCT	GCG	TCC	CGC	TTG	CAA	AGT	SEQ ID NO:33
F21	GCT	GCA	TCC	ACT	TTG	CAA	ACT	SEQ ID NO:35
F22	AGG	GCG	TCC	AGT	TTA	GAA	AGT	SEQ ID NO:37
F23	AGG	GCG	TCT	ACT	TTA	GAC	ATT	SEQ ID NO:39
F26	AAG	GCG	TCT	CGT	TTA	GAA	AAT	SEQ ID NO:41
F133	AGG	GCG	TCT	ACT	TTA	GAC	ATT	SEQ ID NO:43
TA50	GGT	GCA	TCC	ATC	AGG	GCC	CCT	SEQ ID NO:45
TA73	CGT	TCA	TCC	AAT	TTG	AAA	AAA	SEQ ID NO:47

59

```
    Light Chain CDR 3
    Clone
    F3        CAA CAG TAT TCG AGT ACT TTC GGG
              ACC                                  SEQ ID NO:49
 5  F14       CAG CAT TAT AAG AGC GCA CCT CCG
              AGG CAC ACT                          SEQ ID NO:51
    F15       CAA CAG AGT CAT AGA ACC CCG TAC
              ACT                                  SEQ ID NO:53
    F19       CAA CAG AGT TCC GTT AGT CGG ACG      SEQ ID NO:55
10  F21       CAG CAC GCT TAC GCT TTC CCT CGG
              ACG                                  SEQ ID NO:57
    F22       CAG CAC TAT AAC ACT AAC TCT CCG
              CTC ACT                              SEQ ID NO:59
    F23       CAA CAG TAT AGT CGT TAT CCG CTC
15            ACT                                  SEQ ID NO:61
    F26       CAA CTA TAT GAT AGT CAT TCC GCG
              ATG TAC ACT                          SEQ ID NO:63
    F133      CAA CAG TAT AGT CGT TAT CCG CTC
              ACT                                  SEQ ID NO:65
20  TA50      CAG CAG TAT GGT AGG TCA CCT CGG
              GCG                                  SEQ ID NO:67
    TA73      CTG CAG GAT TTC AAT TAT CCC CGG
              ACG                                  SEQ ID NO:69
```

60

Table VI. Amino Acid Sequences of CDR Regions in the Heavy Chains of Fab Fragments Isolated by the Selective Immobilization Filter Lift Technique

Heavy Chain CDR 1
Clone

F3	S Y S M N							SEQ ID NO:72
F14	D Y A I H							SEQ ID NO:74
F15	T Y W M S							SEQ ID NO:76
F19	S S L W W S							SEQ ID NO:78
F21	R Y Y M N							SEQ ID NO:80
F22	S N R A T W N							SEQ ID NO:82
F23	S Y A M N							SEQ ID NO:84
F26	F S N Y D L N							SEQ ID NO:86
F133	S A W L S							SEQ ID NO:88
TA50	S Y W I S							SEQ ID NO:90
TA73	T Y G L S							SEQ ID NO:92

Heavy Chain CDR 2
Clone

F3	Y F S S S G T T I Y Y A D S V K G
	SEQ ID NO:94
F14	F I R S E A F G G T A D Y A A S V K G
	SEQ ID NO:96
F15	N I N Q D G S E K Y Y V D S V E G
	SEQ ID NO:98
F19	E M Y H S G S T K Y N P P S R V
	SEQ ID NO:100
F21	I I N P S G D R T R Y A Q K F Q G
	SEQ ID NO:102
F22	R T Y Y R S E W F N D Y A V F V Q S
	SEQ ID NO:104
F23	Y I S S R G S T I Y Y A D S V K G
	SEQ ID NO:106
F26	T F T G S G D N T Y Y A D S V K G
	SEQ ID NO:108
F133	N I K P D G S D K Y Y V D S V K G
	SEQ ID NO:110
TA50	R I D P S D S Y T N Y S P S F Q G
	SEQ ID NO:112
TA73	W I K P Y N G Y T K Y A Q R L Q D
	SEQ ID NO:114

```
    Heavy Chain CDR 3
    Clone
    F3         G R K T S R Y T L R R F R S I W F D Y
                                                        SEQ ID NO:116
 5  F14        V G R P G E D F                          SEQ ID NO:118
    F15        D P P R Y F G S G S W E                  SEQ ID NO:120
    F19        A S Y T N K W T P I A Y F E Y            SEQ ID NO:122
    F21        P L V Q R D T F Q E                      SEQ ID NO:124
    F22        V P Q V E V P Y                          SEQ ID NO:126
10  F23        F M N R D G P P K R V F D Y              SEQ ID NO:128
    F26        G V S T M V R G L T                      SEQ ID NO:130
    F133       A G P L R I R Y D G F D F                SEQ ID NO:132
    TA50       G R K T S R Y T L R R S E A S G F D Y
                                                        SEQ ID NO:134
15  TA73       D A H R G L V G A T A N F H Y            SEQ ID NO:136
```

62

Table VII. Nucleotide Sequences of CDR Regions in the Heavy Chains of Fab Fragments Isolated by the Selective Immobilization Filter Lift Technique

Heavy Chain CDR 1
Clone

F3	TCC TAT AGC ATG AAC	SEQ ID NO:71
F14	GAC TAT GCT ATA CAT	SEQ ID NO:73
F15	ACC TAT TGG ATG AGC	SEQ ID NO:75
F19	AGT CTT TGG TGG AGT	SEQ ID NO:77
F21	AGG TAT TAT ATG AAC	SEQ ID NO:79
F22	AGC AAT CGT GCT ACG TGG AAC	SEQ ID NO:81
F23	AGC TAT GCC ATG AAT	SEQ ID NO:83
F26	TTT AGT AAT TAT GAT CTG AAC	SEQ ID NO:85
F133	AGC GCC TGG CTG AGC	SEQ ID NO:87
TA50	AGC TAC TGG ATC AGC	SEQ ID NO:89
TA73	ACG TAT GGT CTC AGC	SEQ ID NO:91

Heavy Chain CDR 2
Clone

F3	TAT TTT AGT AGT AGT GGG ACC ACC ATA TAT TAC GCA GAC TCT GTG AAG GGC	SEQ ID NO:93
F14	TTC ATT AGA AGT GAG GCC TTT GGT GGG ACA GCA GAT TAC GCC GCG TCT GTG AAA GGC	SEQ ID NO:95
F15	AAC ATA AAC CAA GAT GGA AGT GAG AAA TAC TAT GTG GAC TCT GTG GAG GGC	SEQ ID NO:97
F19	GAA ATG TAT CAT AGT GGG AGC ACC AAA TAC AAC CCT CCC TCA AGA GTT	SEQ ID NO:99
F21	ATT ATC AAC CCT AGT GGT GAT AGA ACA AGA TAC GCA CAG AAG TTC CAG GGC	SEQ ID NO:101
F22	AGG ACA TAC TAC AGG TCC GAG TGG TTT AAT GAT TAT GCA GTA TTT GTG CAA AGT	SEQ ID NO:103
F23	TAC ATT AGT AGT CGT GGT AGT ACC ATA TAC TAC GCT GAC TCT GTG AAG GGC	SEQ ID NO:105
F26	ACT TTT ACT GGT AGT GGT GAC AAC ACA TAC TAC GCA GAC TCC GTG AAG GGC	SEQ ID NO:107
F133	AAC ATA AAG CCA GAT GGA AGT GAC AAA TAT TAT GTG GAC TCT GTG AAG GGC	SEQ ID NO:109
TA50	AGG ATT GAT CCT AGT GAC TCT TAT ACC AAC TAC AGC CCG TCC TTC CAA GGC	SEQ ID NO:111
TA73	TGG ATC AAG CCT TAC AAT GGT TAT ACA AAG TAT GCA CAG CGC CTC CAG GAT	SEQ ID NO:113

63

Heavy Chain CDR 3
Clone
F3   GGA AGG AAG ACC TCT CGA TAT ACT
            TTG CGA CGT TTC AGA AGT ATC TGG
5   TTT GAC TAC   SEQ ID NO:115
F14   GTC GGG CGC CCG GGT GAG GAC TTT   SEQ ID NO:117
F15   GAT CCC CCA CGA TAC TTT GGT TCG
            GGG AGT TGG GAA   SEQ ID NO:119
F19   GCC AGT TAT ACC AAC AAA TGG ACC
10   CCC ATT GCC TAC TTT GAA TAC   SEQ ID NO:121
F21   CCC CTG GTT CAA CGG GAT ACT TTC
            CAG GAA   SEQ ID NO:123
F22   GTC CCA CAG GTA GAG GTG CCT TAT   SEQ ID NO:125
F23   TTT ATG AAT AGA GAT GGC CCC CCG
15   AAG AGG GTG TTT GAC TAC   SEQ ID NO:127
F26   GGG GTT TCG ACT ATG GTT CGG GGA
            CTG ACC   SEQ ID NO:129
F133   GCT GGC CCA CTC CGT ATC GGG TAT
            GAT GGT TTT GAT TTT   SEQ ID NO:131
20 TA50   GGA AGG AAG ACC TCT CGA TAT ACT
            TTG CGA CGT TCA GAA GCA TCT GGT
            TTC GAC TAC   SEQ ID NO:133
TA73   GAT GCC CAT CGC GGT CTA GTG GGA
            GCT ACA GCA AAC TTT CAC TAC   SEQ ID NO:135
25
_____

        Using the selective immobilization filter lift
technique, a large number of human Fab fragments produced
in an expression library were identified. The technique
allowed the screening of a large number of clones. DNA
30 sequencing of selected numbers of isolated Fab clones
revealed that a variety of genetically distinct Fab
fragments were identified.

        These results show that the selective
immobilization filter lift technique allows
35 identification of a large number of distinct human Fab
fragments.

EXAMPLE VI

Characterization of Tumor Cell Specific Human Fab
Fragments Identified by Selective Immobilization on a
Solid Support

5   This example demonstrates cell specific
reactivity of Fab clones with respect to fixed and live
tumor cell lines.

           Fab fragments identified using the selective
immobilization filter lift technique were purified as
10   described in Example V. Various cell lines were fixed in
paraformaldehyde as described in Example V and incubated
with increasing concentrations of purified Fab fragments
(2.5 x 10^{-1} to 3 x 10^{1} μg/ml). Unbound Fab was removed
and fixed cells were incubated with alkaline phosphatase-
15   conjugated anti-human kappa antibody as described in
Example V. Following incubation with color reagents, the
OD_{560} was measured.

           In Figure 4A, the titration curve of purified
Fab F15 is shown on two breast carcinoma cell lines
20   (H3396 and H3464) and GM05658 normal human fibroblasts.
Fab F15 shows specific binding to both breast cell lines
but not to normal fibroblasts. In Figure 4B, the
titration curve of Fab F133 is shown with the same two
breast cell lines and GM05658 normal human fibroblasts.
25   Fab F133 shows specific binding for the H3396 cells used
for making a screening extract. However, Fab F133 does
not bind to the second breast cell line or to the normal
fibroblasts.

           Purified Fab fragments were also characterized
30   with respect to binding to live tumor cells by FACS
analysis on a Becton Dickinson FACSort system. Purified
Fab F3 was compared to a non-specific Fab fragment on
H3396 cells (see Figure 5A). Incubation of H3396 cells

65

with Fab F3 caused a shift in H3396 cells, indicating
reactivity with a cell surface antigen, compared to cells
incubated with non-specific Fab. The peak of Fab F3
shifted cells is sharp, indicating that the population of
5   H3396 cells are relatively homogeneous in their
expression of antigen.

A similar FACS analysis was performed with Fab
F15. As shown in Figure 5B, Fab F15 resulted in a larger
shift in H3396 cells relative to non-specific Fab than
10   was observed with Fab F3, indicating that Fab F15 reacts
with a cell surface antigen. The Fab F15 shifted peak is
broader than that observed with Fab F3, consistent with a
more heterogeneous expression of the Fab F15 antigen on
H3396 cells.

15   A FACS analysis was also performed with Fab
F40. FAB F40 was identified using membrane extracts of
the breast cell line H3396. Interestingly, Fab F40 is
cross-reactive with the colon carcinoma cell line H3719
(see Figure 5C). Fab F40 results in a shift in H3719
20   cells relative to control non-specific Fab, indicating
that Fab F40 recognizes a cell surface antigen on the
colon carcinoma cell line. The Fab F40 peak is somewhat
broad, indicating that expression of the F40 antigen is
more heterogeneous. Thus, Fab F40 recognizes a cell
25   surface antigen on two different types of tumor cells.

The selective immobilization filter lift
technique allowed isolation of a large number of human
Fab fragments with specificity for fixed and live tumor
cells. While some Fab fragments are specific for breast
30   carcinoma cells or are specific for a particular breast
cell line, other Fab fragments cross react with a colon
carcinoma cell line. These Fab fragments displayed
specific binding on tumor cells but did not bind to
normal fibroblasts.

66

These results indicate that the selective
immobilization filter lift technique allows
identification of human Fab fragments with specificity
for tumor cells.

5   All journal article and references provided
above, in parentheses or otherwise, whether previously
stated or not, are incorporated herein by reference in
their entirety.

Although the invention has been described with
10 reference to the examples provided above, it should be
understood that various modifications can be made without
departing from the spirit of the invention. Accordingly,
the invention is limited only by the claims.

What is claimed is:

1.   A method for identifying a binding molecule having selective affinity for a ligand, comprising the steps of:

5   a)   selectively immobilizing a diverse population of binding molecules to a solid support;

b)   simultaneously contacting said diverse population immobilized on said solid support with two or more ligands; and

10   c)   determining at least one binding molecule which selectively binds to one or more of said ligands.

2.   The method of claim 1, further comprising determining the sequence of said binding molecule determined in step (c).

15   3.   The method of claim 1, further comprising the step of characterizing said ligand which binds to said binding molecule.

4.   The method of claim 1, wherein said population of binding molecules is produced in an
20   expression library.

5.   The method of claim 4, wherein said expression library is an antibody library.

6.   The method of claim 1, wherein said ligands are polypeptides.

25   7.   The method of claim 6, wherein said polypeptides are cell surface polypeptides.

68

8. The method of claim 6, wherein said polypeptides are tumor antigens.

9. The method of claim 1, wherein said ligands are in a cell lysate.

5   10. A method for identifying an antibody having selective affinity for a tumor antigen, comprising the steps of:

a)   selectively immobilizing a diverse population of antibodies to a solid support;

10   b)   simultaneously contacting said diverse population immobilized on said solid support with two or more tumor antigens; and

c)   determining at least one antibody which selectively binds to one or more of said tumor antigens.

15   11. The method of claim 10, wherein said population of antibodies are human antibodies or functional fragments thereof.

12. The method of claim 10, wherein said population of antibodies is produced in an expression
20 library.

13. An isolated binding polypeptide selective for a tumor antigen, said binding polypeptide an antibody fragment selected from the group of antibody fragments consisting of F3, F13, F14, F15, F19, F21, F22, F23, F26,
25 F30, F31, F32, F33, F34, F35, F36, F37, F38, F40, F41, F42, F46, F49, F50, F52, F54, F55, F58, F63, F66, F67, F68, F69, F70, F72, F74, F76, F78, F79, F80, F81, F84, F85, F86, F93, F99, F104, F111, F112, F118, F126, F129, F130, F132, F133, F134, F135, F136, F138, F151, F158,

69

F160, F174, F176, F177, F184, F186, F191, F197, F200,
F202, F203, F207, F208, F212, F214, F217, F224, F231,
F236, F238, TA50 and TA73.

5   14.   An isolated binding polypeptide selective
for a tumor antigen, said binding polypeptide an antibody
fragment selected from the group of antibody fragments
consisting of F3, F14, F15, F19, F21, F22, F23, F26,
F133, TA50 and TA73.

10   15.   The antibody fragment of claim 14,
comprising substantially an amino acid sequence selected
from the group consisting of the amino acid sequences
referenced as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ
ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ
15   ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ
ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ
ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ
ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ
ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ
ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ
20   ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ
ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ
ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ
ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ
ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106,
25   SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID
NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ
ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128,
SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, and SEQ ID
NO:136.

30   16.   An isolated nucleic acid molecule encoding
an antibody fragment, comprising a nucleotide sequence
encoding substantially an amino acid sequence selected

from the group consisting of the amino acid sequences
referenced as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ
ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ
ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ

5   ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ
ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ
ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ
ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ
ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ

10   ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ
ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ
ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ
ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ
ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106,

15   SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID
NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ
ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128,
SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, and SEQ ID
NO:136.

20   17.   The isolated nucleic acid molecule of
claim 16, comprising a nucleotide sequence selected from
the group consisting of the nucleotide sequences
referenced as SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ
ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ

25   ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ
ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ
ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ
ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ
ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ

30   ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ
ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ
ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ
ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ
ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ

71

ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105,

SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID

NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ

ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127,

5   SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID

NO:135.

        18.   A Complementarity Determining Region (CDR)

or functional fragment thereof of an antibody selective

for a tumor antigen, said CDR derived from an antibody

10 fragment selected from the group consisting of F3, F13,

F14, F15, F19, F21, F22, F23, F26, F30, F31, F32, F33,

F34, F35, F36, F37, F38, F40, F41, F42, F46, F49, F50,

F52, F54, F55, F58, F63, F66, F67, F68, F69, F70, F72,

F74, F76, F78, F79, F80, F81, F84, F85, F86, F93, F99,

15 F104, F111, F112, F118, F126, F129, F130, F132, F133,

F134, F135, F136, F138, F151, F158, F160, F174, F176,

F177, F184, F186, F191, F197, F200, F202, F203, F207,

F208, F212, F214, F217, F224, F231, F236, F238, TA50 and

TA73.

20   19.   A Complementarity Determining Region (CDR)

or functional fragment thereof of an antibody selective

for a tumor antigen, said CDR derived from an antibody

fragment selected from the group consisting of F3, F14,

F15, F19, F21, F22, F23, F26, F133, TA50 and TA73.

25   20.   The CDR of claim 19, comprising

substantially an amino acid sequence selected from the

group consisting of the amino acid sequences referenced

as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12,

SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20,

30 SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28,

SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36,

SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44,

SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52,

SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60,

SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68,

SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76,

SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84,

5 SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92,

SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100,

SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID

NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ

ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122,

10 SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID

NO:130, SEQ ID NO:132, SEQ ID NO:134, and SEQ ID NO:136.

21. An isolated nucleic acid molecule encoding

a CDR, comprising a nucleotide sequence encoding

substantially an amino acid sequence selected from the

15 group consisting of the amino acid sequences referenced

as SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12,

SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20,

SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28,

SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36,

20 SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44,

SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52,

SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60,

SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68,

SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76,

25 SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84,

SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92,

SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100,

SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID

NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ

30 ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122,

SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID

NO:130, SEQ ID NO:132, SEQ ID NO:134, and SEQ ID NO:136.

22. The isolated nucleic acid molecule of
claim 21, comprising a nucleotide sequence selected from
the group consisting of the nucleotide sequences
referenced as SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ
5   ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ
ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ
ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ
ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ
ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ
10 ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ
ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ
ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ
ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ
ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ
15 ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ
ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105,
SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID
NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ
ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127,
20 SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, and SEQ ID
NO:135.
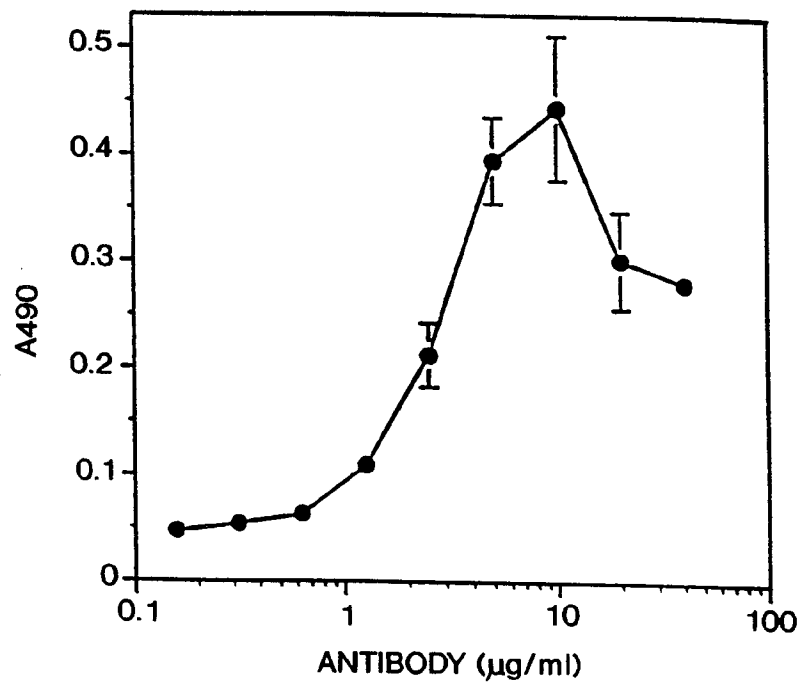
FIG. 1A
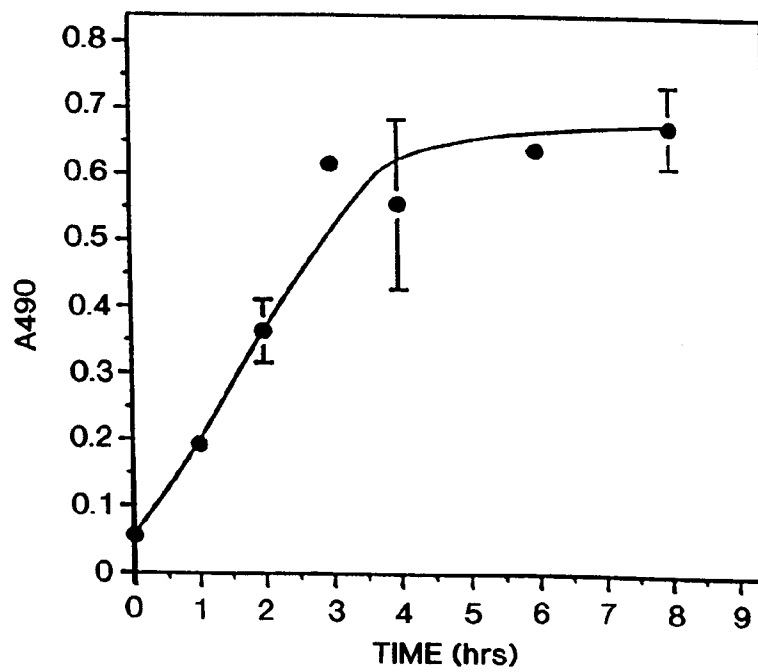
FIG. 1B
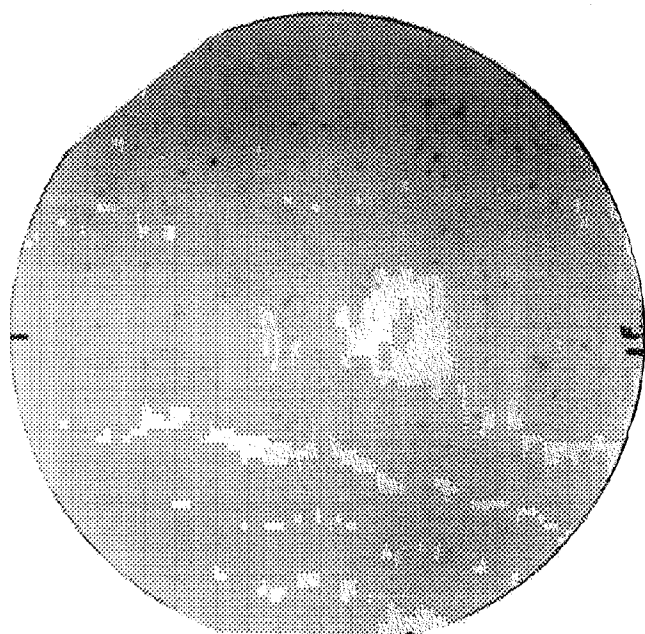
Normal Lift

FIG. 2B
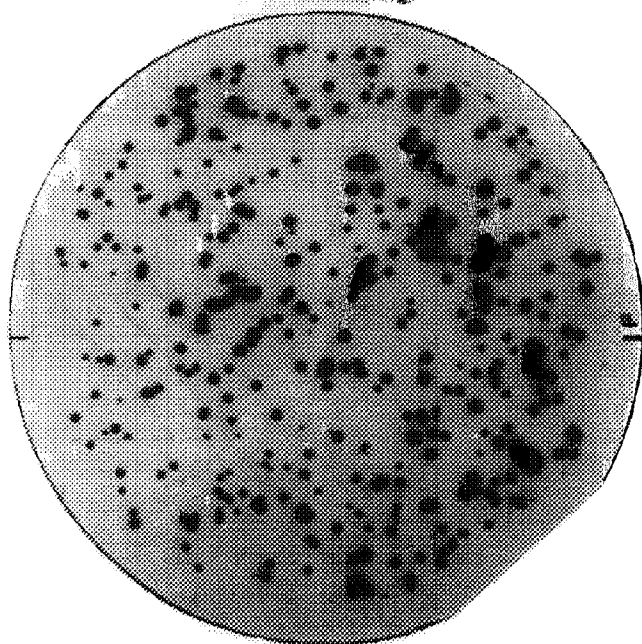
Capture Lift

FIG. 2A
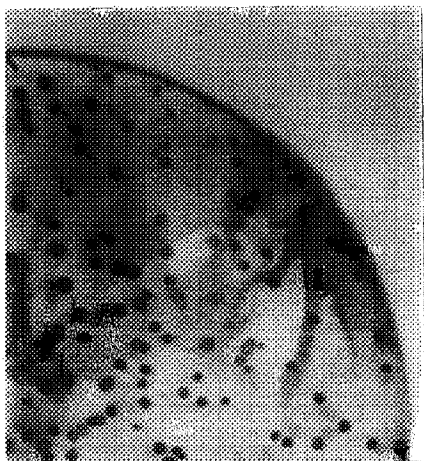
α-fd bacteriophage
FIG. 3A
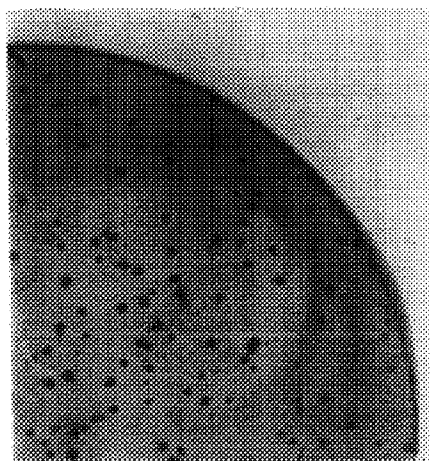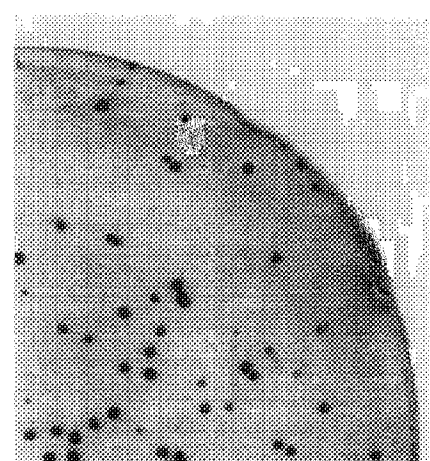

biotinylated membranes
FIG. 3B

NORMAL LIFTS

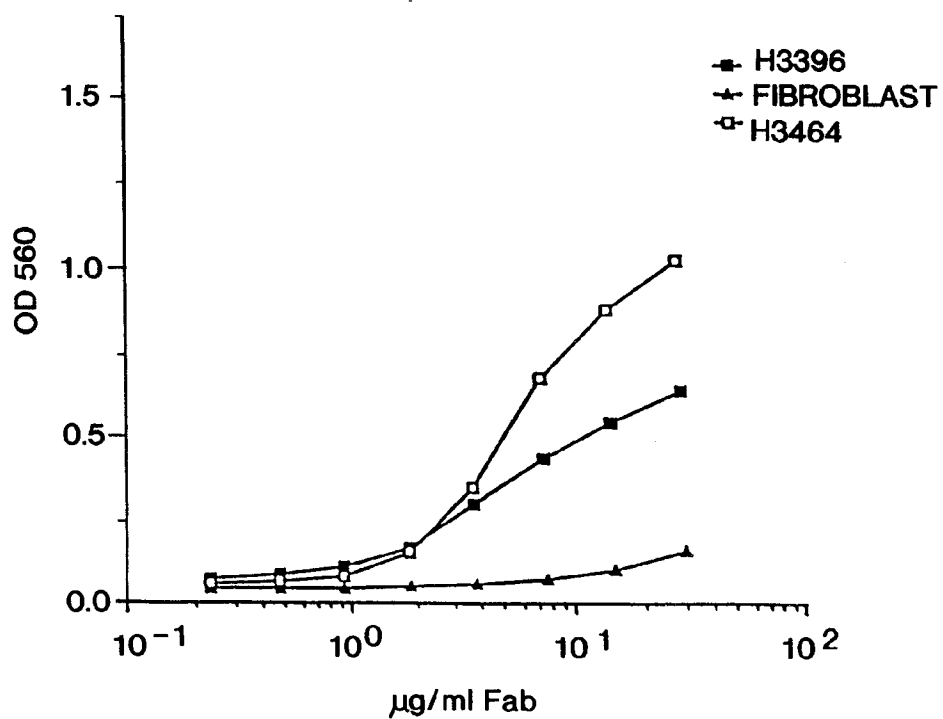Lewis Y-HRP
FIG. 3C

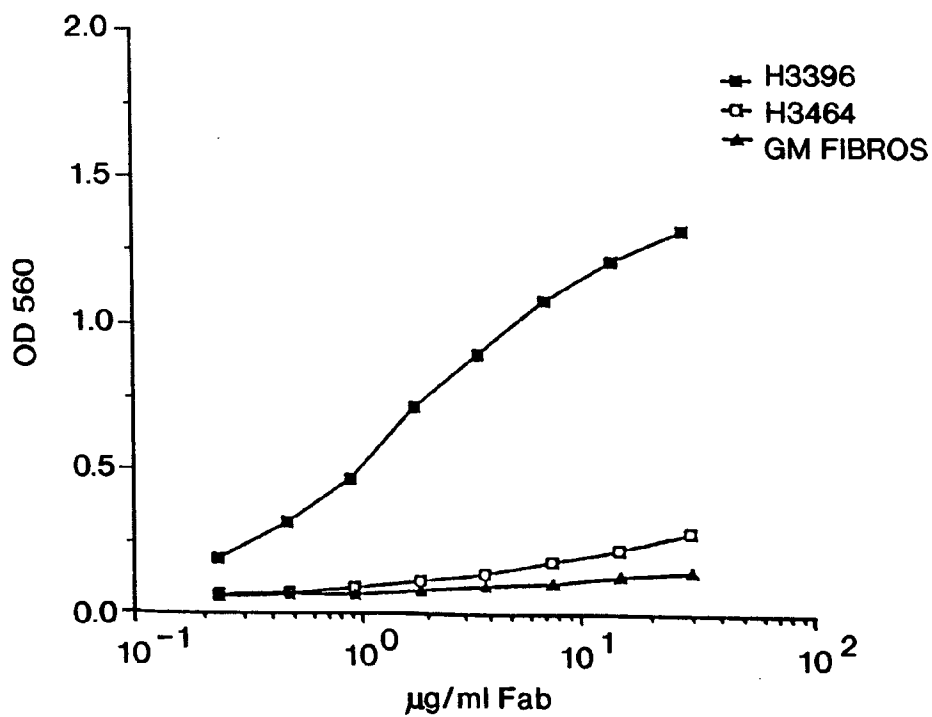biotinylated membranes
FIG.3D

CAPTURE LIFTS

FIG. 4A

F133

FIG. 4B

5/5

FIG. 5A

FIG. 5B

FIG. 5C